DESIGN, DEVELOPMENT AND EVALUATION OF METRONIDAZOLE BENZOATE LOADED PRONIOSOMES FOR TOPICAL DELIVERY

Dissertation submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the requirement of the degree of

MASTER OF PHARMACY

(Pharmaceutics)

OCTOBER- 2016

DEPARTMENT OF PHARMACEUTICS

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KOVAI ESTATE, KALAPATTI ROAD, COIMBATORE- 641048

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CERTIFICATE

This is to certify that this dissertation work entitled **"DESIGN, DEVELOPMENT AND EVALUATION OF METRONIDAZOLE BENZOATE LOADED PRONIOSOMES FOR TOPICAL DELIVERY"** was carried out successfully by **Reg.no:261410911.** The work mentioned in the dissertation was carried out at the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore - 641048, under the guidance of **DR. C Sankar,M.Pharm.,P.hD.,** for the partial fulfillment for the Degree of Master of Pharmacy and is forwarded to The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

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Place: Coimbatore

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DECLARATION

I do hereby declare that this dissertation entitled **"DESIGN, DEVELOPMENT AND EVALUATION OF METRONIDAZOLE BENZOATE LOADED PRONIOSOMES FOR TOPICAL DELIVERY"** submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** was done by me under the guidance of, **DR.C Sankar, M.Pharm.,Ph.D.,** Professor, Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, during the year 2015-2016.

(Reg.no:261410911)

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"DESIGN, DEVELOPMENT AND EVALUATION OF METRONIDAZOLE BENZOATE LOADED PRONIOSOMES FOR TOPICAL DELIVERY"** submitted by **Reg.no:261410911** to The Tamil Nadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore, and was evaluated by us during the academic year 2015– 2016.

Examination Centre: KMCH College of Pharmacy, Coimbatore – 48.

Date:

Internal Examiner **External Examiner**

Convener of Examination

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Above all I dedicate myself before the unfailing presence of **God Almighty** throughout the way of my success.

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CONTENTS

ABBREVIATIONS

e.g	Example
i.e	That is
%	Percentage
&	And
kg	Kilogram
mg	Milligram
nm	Nanometer
mm	Millimeter
µg	Microgram
FFEM	Freeze Fracture Electron Microscopy
SAXS	Small Angle X-Ray Scattering
HLB	Hydrophilic –Lipophilic Balance
CPP	Critical Packing Parameter
TDDS	Transdermal Drug Delivery System
HPMC	Hydroxy Propyl Methyl Cellulose
Avg	Average
Hrs	Hours
RPM	Revolutions Per Minute
CR	Cumulative Release
Conc	Concentration
R^2	Regression coefficient
HTN	Hypertension
FPM	First Pass Metabolism
B.A	Bioavailability
PPB	Plasma Protein Binding
V_d	Volume of distribution

ADR	Adverse drug reaction
SEM	Scanning Electron Microscopy
E E	Entrapment efficiency
MB	Metronidazole benzoate
n	Slope
ICH	International Conference on Harmonisation
RH	Relative Humidity
FT-IR	Fourier transform infra red spectroscopy

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ABSTRACT

Nowadays, vesicles have become the carrier of choice in drug delivery. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both hydrophilic and lipophilic drugs. Proniosomes are one of the important novel drug delivery carriers of various drug molecules. The design of the present investigation was to prepare and develop Metronidazole Benzoate loaded proniosomes with different ratios of cholesterol and non-ionic surfactants to achieve prolonged release of drug on topical administration. A total of fourteen formulations were prepared by slurry method and evaluated for angle of repose, drug content, particle size, hydration rate, encapsulation efficiency, *in-vitro* release studies& *ex-vivo* drug permeation studies. The optimized formulation of proniosome was incorporated into transdermal patch and was subjected to various evaluation studies. The *in-vitro* diffusion and kinetic study of the patch showed a release of 80.71% over 12hrs and fitted into zero order and non-fickian diffusion mechanism. It could be concluded that the formulationF_{12} having surfactant: cholesterol concentration as 1:1 was the best formulation. The SEM image as well as the FT-IR spectrum of the optimized formulation was taken.

Keywords: Metronidazole benzoate, Proniosome, Slurry method, *In vitro* drug release, *Ex vivo* permeation study, SEM.

INTRODUCTION

The skin is the single largest organ of the body. Skin serves as a barrier for the drugs to pass through it. Percutaneous absorption involves the passage of the drug molecule from the skin surface into the stratum corneum under the influence of the concentration gradient and its subsequent diffusion through the stratum corneum and underlying epidermis, through the dermis and into the blood circulation. The resistance provided by the skin is the major rate limiting step in the percutaneous absorption.[1]

Various techniques have been employed to weaken the skin barrier and deliver the drug into the body through the intact skin. Some of the techniques include iontophoresis, sonophoresis, electroporation, use of chemical enhancers and microneedles. These methods are not used now because of limited efficacy, skin irritation, complexity in usage and high cost. Due to this many elastic lipid systems like niosomes, liposomes ,transfersomes, niosomes and proniosomes were developed in order to penetrate deeply and easily into skin. These vesicles squeeze themselves through the pores of the stratum corneum due to their highly flexible membrane.[2]

VESICULAR DRUG DELIVERY SYSTEM [3]

Vesicular drug delivery systems have gained a lot of interest as a carrier for advance drug delivery. Encapsulation of the drug in carrier structures is one such system, which delays the drug release and reduces the toxicity by selective uptake. The drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have proved distinct advantages over conventional dosage forms because they can act as drug reservoir and can carry both hydrophilic and hydrophobic drugs. The vesicles dispersed in aqueous systems may suffer from some problems like degradation by hydrolysis or oxidation, sedimentation, aggregation or fusion of liposomes during storage. Two novel approaches adopted to avoid these problems were to develop the proliposomes and to develop niosomes using non-ionic surfactants alternative to phospholipids in preparing vesicles. Though proliposomes are advanced over conventional liposomes some physical instability still persist, so a vacuum or nitrogen atmosphere is optional during preparation and storage to prevent the oxidation of phospholipids. In later approach, niosomes exhibit good chemical stability during storage but the aqueous suspension of niosomes exhibit physical stability problems like aggregation, fusion, leaking of entrapped drugs or hydrolysis of encapsulated drugs etc. The latest

approach in the field of vesicular delivery is to combine the two previously mentioned techniques by extending the pro-vesicular approach to niosomes through the formation of "proniosomes" which are converted to niosomes.

TYPES OF VESICULAR DRUG DELIVERY SYSTEM [4]

1. Liposomes
2. Virosomes
3. Niosomes
4. Proniosomes
5. Transfersomes
6. Proteasomes
7. Sphingosomes
8. Ethosomes

LIPOSOME [5]

They are artificially prepared vesicles composed of lipid bilayer. They can be prepared by disrupting the biological membrane. They are composed of natural phospholipids and may also contain lipid chains with surfactant properties.

VIROSOME[4]

They consists of uni lamellar phospholipid membrane which is either a mono or bi layer vesicle incorporating virus derived proteins to allow virosomes to fuse with target cells.

NIOSOME[4]

They are non- ionic surfactant based liposome. Niosomes are mostly formed by cholesterol incorporation. They are structurally similar to liposomes.

TRANSFERSOME[4]

A transfersome carrier is an artificial vesicle designed to be such as a cell vesicle or a cell engage in exocytosis & thus suitable for controlled & potentially targeted drug delivery.

PROTEASOME[4]

They are cytoplasmic organelles responsible for degrading endogenous proteins because of the presence of ubiquitin conjugated to the targeted protein's lysine residue.

SPHINGOSOME[6]

They are bilayered vesicles in which an aqueous volume is entirely enclosed by lipid bi- layer membrane mainly composed of natural or synthetic sphingolipid.

ARCHAESOME[4]

These are liposomes made from polar ether lipids of *Archaea.*

ETHOSOME [7]

They are modified forms of liposomes which have high ethanol content. They are flexible,malleable vesicles adapted for enhanced delivery of active agents.

PRONIOSOME[8]

Proniosomes are dry, free flowing, granular product that could be hydrated immediately before use by agitation in aqueous media to form the niosomal suspension.

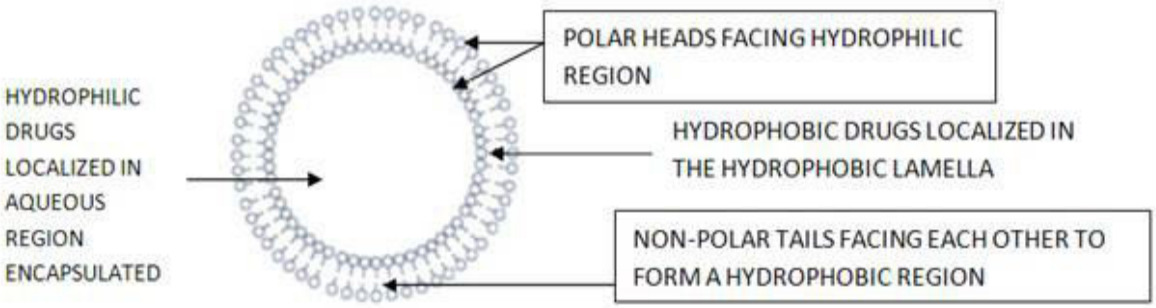


Figure1: Schematic diagram of proniosome

ADVANTAGES OF PRONIOSOMES [3]

- The problems of physical stability like fusion, aggregation, sedimentation, &leakage on storage can be reduced.
- Hydrolysis of encapsulated drug which limits the shelf life can be avoided.
- Since it's a dry formulation provide convenience in storage and transportation.
- Provides drug delivery with improved bioavailability &reduced side effects.
- Both hydrophilic and hydrophobic drugs can be entrapped.
- Shows controlled and sustained release of drugs due to depot formation.
- Biodegradable, biocompatible & non-immunogenic to the body.

INTERACTION BETWEEN PRONIOSOME AND SKIN [9]

There is a direct contact of the proniosomal formulation with the skin after it is applied. Since the proniosomes are made of non-ionic surfactants, it is important to study the possible interactions between the non-ionic surfactants and the skin. The non-ionic surfactants are amphipathic molecules consisting of hydrophobic (alkylated phenol derivatives, fatty acids, long chain linear alcohol etc) & a hydrophilic part (ethylene oxide chains of variable length). Skin consists of a range of bio active materials like membrane phospholipids, proteins, amino acids, peptides etc.

Surfactants are known to increase the permeability of vesicles and phospholipid membranes, causing low molecular mass compounds to leak. The non-ionic surfactants are included in the pharmaceutical formulations to increase their stability, solubility & permeation.

FACTORS AFFECTING NATURE OF PRONIOSOMES

- Hydration temperature
- Choice of main surfactant
- Nature of drug
- Nature of membrane additives
- Size reduction techniques
- Addition of kinetic energy

MECHANISM OF DRUG PERMEATION FROM VESICLES THROUGH SKIN[19]

The following types of vesicle-skin interactions are observed during *in-vitro* studies using human skin.

1. Absorption & fusion of vesicles onto skin surface leads to increase in thermodynamic activity gradient of the drug at the interface, which acts as a driving force for absorption of lipophilic drugs across the stratum corneum .
2. Modification in the structure of the stratum corneum is also a type of interaction which involves the ultra structural changes in the intracellular lipid region of the skin & its deeper layers which is revealed by Freeze Fracture Electron Microscopy (FFEM) & Small angle X- ray scattering (SAXS).
3. Bilayer present in the niosomes act as rate limiting barrier for drugs.
4. Proniosomes contain both non-ionic surfactant & phospholipids, both can act as penetration enhancer & are useful in increasing penetrability of many drugs.
5. The penetration enhancer effect of vesicles to reduce stratum corneum barrier properties.

MECHANISM OF VESICLE FORMATION IN PRONIOSOMES[19]

Non-ionic surfactants have the ability to form bilayer vesicles which depends on the HLB of surfactant & also on critical packing parameter. CPP can be defined as the relationship between structure of surfactant including size of hydrophilic head group & length of hydrophobic alkyl chain in the ability to form vesicles is described as

CPP= u/lca (1)

Where, u= the hydrophilic group volume

lc =critical hydrophobic group length

a= area of hydrophilic head group

When the value of CPP is between 0.5 to 1, then the surfactant is likely to form vesicles. CPP below 0.5(indicates that there is high contribution from hydrophilic head groups) gives spherical micelles & value of CPP above 1 (indicates that there is high contribution from the hydrophobic group) gives inverted micelles which on later stage gives precipitation. Spans are most widely used in proniosomal preparation.

All grades of span have same head group but are differentiated on basis of alkyl chain. The entrapment efficiency increases as the alkyl chain length increases.

Span60 (C18) > Span40 (C16) > Span20 (C12) > Span80 (C18).

Span 60 &80 have same head group but there is difference in the alkyl chain of span80 which is unsaturated.

The low entrapment efficiency of span80 may be due to introduction of double bond to its paraffin chain. Cholesterol also provides stability to the bilayer membrane by increasing gel liquid transition temperature of vesicles &also attributes to high HLB value & small CPP.

CATEGORIES OF PRONIOSOMES

- Dry Granular type of proniosome
  - *Sorbitol based proniosomes
  - *Maltodextrin based proniosomes
- Liquid crystalline proniosome

DRY GRANULAR PRONIOSOME [10]

Dry granular type of proniosomes involves the coating of water-soluble carrier such as sorbitol and maltodextrin with surfactant. The result of coating process is a dry formulation in which each water-soluble particle is covered with thin film of surfactant. It is essential to prepare vesicles at a temperature above the transition temperature of the non-ionic surfactant being used in the formulation. These are further categorized as follows

- Sorbitol based proniosome

Sorbitol based proniosomes is a dry formulation that involves sorbitol as the carrier, which is further coated with non-ionic surfactant and is used as niosomes within minutes by addition of hot water followed by agitation. They are normally made by spraying surfactant mixture prepared in organic solvent onto the sorbitol powder and then evaporating the solvent. Since the sorbitol carrier is soluble in organic solvent, the process is required to be repeated till the desired surfactant coating has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves.

- Maltodextrin based proniosome [11]

Maltodextrin is used as the carrier. The principal advantage with this formulation is the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared.

LIQUID CRYSTALLINE PRONIOSOME

When the surfactant molecules are in contact with water, the lipohilic chains of surfactants get transformed into a disordered liquid state called lytorophic liquid crystalline state also called as neat phase. This transformation occurs by increasing the temperature at the kraft point , addition of solvent or use of both the temperature & solvent.

Neat phase or lamellar phase contains bilayer arranged in sheets over one another within the intervening aqueous layer. These liquid crystalline proniosomes act as a reservoir for transdermal delivery of drug.

PRONIOSOMES AS DRUG CARRIERS[8]

The proniosomes are promising drug carriers as they possess greater chemical stability and lack of many disadvantages associated with liposomes. Additional merit is its non –toxic nature due to the use of non-ionic surfactants in its preparation. It does not require special

conditions and precautions for formulation and preparation. They are dry free flowing product which makes them more stable during sterilisation and storage & also ease of transfer, distribution and measuring make them a pronouncing versatile delivery system.

COMPONENTS OF PRONIOSOMES[12]

Surfactants

Surfactants are surface active agents usually organic compounds that are amphiphilic in nature i.e they have have both hydrophobic & hydrophilic groups. They function as solubilizer, wetting agents,emulsifier & permeability enhancer. Only non-ionic surfactans are used for the preparation of proniosomes.

e.g :* Alkyl ethers & alkyl glyceryl ethers- Polyoxyethelene 4 lauryl ether

Polyoxyethelene cetyl ethers

Polyoxyethelene stearyl ethers

*Sorbitan fatty acid esters -Span20,40,60,80

*Polyoxyethelene fatty acid esters -Tween 20,40,60,80

Carrier material

The carrier which is non- toxic, free flowing, poor solubility in the solvent are used for the preparation of proniosome & good aqueous solubility for hydration.

E.g: Maltodextrin, sorbitol, mannitol, spray dried lactose, glucose monohydrate, lactose monohydrate, sucrose stearate.

Membrane stabilizer

Cholesterol &lecithin are mainly used as membrane stabilizer. They influence the stability &permeability of the vesicles. Steroids are components of cell membrane, they bring about significant changes in the bilayer stability, fluidity & permeability. Cholesterol is a naturally occurring steroid used as membrane additive. It stabilises the system by means of electrostatic effects &also by prevention formation of the aggregates. It also leads to change of gel state to liquid state in niosomal system.

Phosphatidylcholine is a major constituent of lecithin. It has low solubility in water & can form liposomes, bilayer sheets, micelles structures depending on hydration & temperature. Depending on the source, there are egg lecithin & soya lecithin. The vesicles composed of soya lecithin are larger in size then the one formed from egg lecithin, due to the difference in intrinsic composition.

Solvent & aqueous phase

Alcohol used in proniosome has a great effect on vesicle size & drug permeation rate. The size of the vesicles formed from different alcohol is different and are in the order: ethanol>propanol>butanol>isopropanol. Ethanol forms the largest sized vesicles due to its greater solubility in water. Ispropanol forms vesicles of smallest size due to its branched chain. Phosphate buffer pH7.4, 0.1% glycerol, hot water is used as the aqueous phase in the preparation of proniosomes.

PREPARATION OF PRONIOSOMES

There are 3 methods of preparation:

- SLURRY METHOD[9]

The carrier was taken in RBF and the entire volume of surfactant dissolved in the organic solvent was added to form slurry. The flask was then attached to the rotary evaporator & vacuum was applied until a dry free flowing powder was obtained. The flask was removed from the evaporator and kept under vacuum overnight. The obtained proniosomal powder was stored in sealed container at4ºC.

- COASERVATION PHASE SEPARATION METHOD[12]

Weighed amounts of surfactant, lipid & drug were taken in a clean dry wide mouthed glass vial &0.5ml alcohol was added to it. All the ingredients were mixed well after warming. The vial was closed with a lid to prevent the loss of solvent. The surfactant mixture was dissolved completely by warming it over a water bath at 60-70ºC. Then the aqueous phase was added & warmed on water bath till a clear solution was formed which was then converted into a proniosomal gel.

- SLOW SPRAY COATING METHOD[9]

This method involves preparation of proniosomes by spraying surfactant in organic solvent onto the carrier (sorbitol) & then evaporating the solvent. Because the sorbitol carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant loading has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to be formed as the carrier dissolves. The resulting niosomes are very similar to those produced by conventional methods and the size distribution is more uniform.

DRUG OF CHOICE[3]

The drug selection should be based on the following assumptions:

- Should have low aqueous solubility.
- Suitable for controlled drug delivery.
- Higher adverse drug reactions.
- Short half-life.

TRANSDERMAL DRUG DELIVERY[20]

Transdermal drug delivery is defined as self-contained, discrete dosage form which when applied to unbroken skin , delivers the drug through the skin in a controlled manner to the systemic circulation. The transdermal patch uses a polymer to control the rate of drug delivery from the reservoir through the skin & into the blood stream. The approach of TDDS is more significant in case of chronic disease like HTN, which require prolonged period of dosing to maintain therapeutic drug concentration.

ADVANTAGES OF TDDS[21]

1. Avoids FPM of drugs
2. Reduction of fluctuations in plasma levels.
3. Reduced plasma drug concentration with decreased side effects.
4. Reduction of dosing frequency & enhancement of patient compliance.
5. Maintains the plasma drug concentration of potent drugs.
6. Termination of therapy is easy at any point of time.
7. Site specific delivery of drug is possible.
8. Self- administration is possible.

LIMITATIONS OF TDDS

a. Not suitable for those which require more than 10mg/day.
b. Patients develop contact dermatitis at the site of application for one or more components, necessitating discontinuation.
c. Only moderately potent drugs can be incorporated.
d. The function of the skin changes from one site to another in the same individual & from individual to individual & with age.

Conditions for drug permeation

Hydration: Hydrated skin is more permeable.

Broken or irritated skin: Drugs can more easily bypass the stratum corneum, increases permeability.

Temperature: Warmer skin is more permeable.

Sunburn: Initially skin is less permeable, after peeling it becomes more permeable.

Psoriasis: Regions exhibit increased or decreased permeability.

Skin as a Site for Drug Infusion[25,26]

The skin of an average adult body covers a surface area of approximately two square meters and receives about one-third of the blood circulating through the body. The skin is a multilayered organ composed of many histological layers. It is generally described in terms of three major tissue layers: the epidermis, the dermis, and the hypodermis. Microscopically, the epidermis is further divided into five anatomical layers with stratum corneum forming the outer most layer of the epidermis, exposing to the external environment.

An average human skin surface is known to contain, on the average, 40-70 hair follicles and 200-250 sweat ducts on each cm^2 of skin area. These skin appendages however actually occupy grossly, only 0.1% of the total human skin surface. Even though the foreign agents, especially the water-soluble ones, may be able to penetrate into the skin via these skin appendages at a rate which is faster than through the intact area of the stratum corneum. This trans-appendage route of percutaneous absorption has, at steady state, a very limited contribution to the overall kinetic profile of transdermal permeation. Therefore, the transdermal permeation of most neutral molecules can, thus, be considered as, a process of passive diffusion through the intact stratum corneum in the interfollicular region**.**

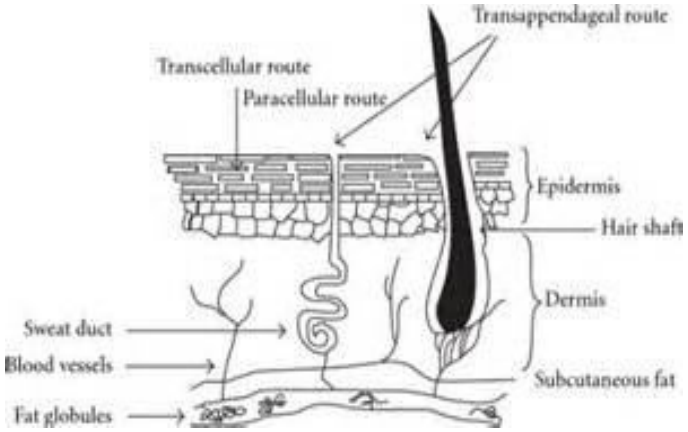


Figure 2: Routes of transdermal delivery of drug

TRANSDERMAL PATCHES[31]

A transdermal patch or skin patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and directly into the bloodstream.

Various drugs which are administered through skin patches include scopolamine (for motion sickness), nicotine (for quitting smoking), estrogen (for menopause and to prevent osteoporosis), nitroglycerin (for angina), lidocaine (to relieve the pain of shingles) and many more drugs.

MECHANISM OF ACTION OF TRANSDERMAL PATCHES[27]

The application of the transdermal patch and the flow of the active drug constituent from the patch to the circulatory system via skin occur through various methods.

1. Iontophoresis

2. Electroporation

3. Ultrasound

4. Microscopic projection

Iontophoresis

It passes a few milli amperes of current to a few cm^2 of skin through the electrode placed in contact with the formulation, which facilitates drug delivery across the barrier. This technique is mainly used for pilocarpine delivery to induce sweating as part of cystic fibrosis diagnostic test.

Electroporation

Electroporation is a technique of application of short, high-voltage electrical pulses to the skin. After electroporation, the permeability of the skin for diffusion of drugs is increased by 4 times. The electrical pulses are believed to form transient aqueous pores in the stratum corneum, through which drug transport occurs.

Ultrasound

Use of ultrasound, particularly low frequency ultrasound, has been shown to enhance transdermal transport of various drugs including macromolecules. It is also known as sonophoresis.

Microscopic projection

In this method, microneedles are used to facilitate transdermal drug transport through the skin. Needles ranging from approximately 10-100 μm in length are arranged in arrays. When pressed into the skin, the arrays make microscopic punctures that are large enough to deliver macromolecules, but small enough that the patient does not feel the penetration or pain. The drug is surface coated on the microneedles to aid in rapid absorption. They are used in development of cutaneous vaccines for tetanus and influenza.

Selection of the drug for transdermal drug delivery system

Before the development of the transdermal drug delivery system of any drug, various pharmacokinetics and pharmacodynamic properties are taken into consideration. Typical requirement for transdermal delivery of drug includes

* Low molecular weight ranging from 500 to 1000 Daltons.

*Low melting point (150-2000F).

*Aqueous solutions neither too acidic nor basic (between 5 and 9 pH units)

* Preferable lipid/water co-efficient i.e. partition coefficient.

*The most important requirement of the drug to be delivered transdermally is demonstrated by need for controlled delivery, such as short half-life and adverse effects associated with other routes or complex oral route or IV dose regimen.

* Drugs which get extensively metabolized in the hepatic first pass effect.

BASIC COMPONENTS OF TRANSDERMAL DRUG DELIVERY SYSTEMS[28]

The components of transdermal devices include:

1. Polymer matrix or matrices

2. The drug

3. Permeation enhancers

4. Other excipients

Polymer matrix

The polymer controls the release of the drug from the device. The following are the criteria should be satisfied for a polymer to be used in the transdermal system:

* The polymer should be stable, non reactive with the drug, easily manufactured and fabricated into desired product and inexpensive.

*The polymer and its degradation products must be non toxic to the host.

* The mechanical properties of the polymer should not deteriorate excessively when large amounts of active agent are incorporated in to it.

e.g: Cellulose derivatives, Polybutadiene, Neoprene, Polyamides etc.

Drug

The following are some of the desirable properties of a drug for tansdermal delivery:

* The drug should have a molecular weight less than 1000 Daltons.

* The drug should have affinity for both- lipophilic and hydrophilic phases.

* The drug should have low melting point.

* The drug should be potent with a daily dose of the order of a few mg/day.

* The half life of the drug should be short.

* The drug must not induce a cutaneous irritation or allergic response.

* Drug which degrade in the GI tract or inactivated by hepatic first-pass effect are suitable candidates for transdermal delivery.

* Drugs which have to be administered for a long period of time or which cause adverse effects to non-target tissues can also be formulated for transdermal delivery.

Permeation enhancers

These are the compounds which promote skin permeability by altering the skin & increase the flux of a desired penetrant.

The flux,J, of drugs across the skin can be written as:

$$J = D\, dc/dx \qquad (2)$$

D = Diffusion coefficient

C =Concentration of the diffusing species.

Permeation enhancers are hypothesized to affect one or more of these factors to achieve skin penetration enhancement. A large number of compounds have been investigated for their ability to enhance stratum corneum permeability. These may be conveniently classified under the following main headings:

a. Solvents

The compounds increase penetration possibly by swelling the polar pathway and by fluidizing lipids.

E.g : Methanol, Ethanol, Dimethyl sulfoxide, Dimethyl acetamide, Propylene glycol, Glycerol.

b. Surfactants

These compounds are proposed to enhance polar pathway transport, especially of hydrophilic drug. The stability of a surfactant to alter penetration is a function of the polar head group and the hydrocarbon chain length. These compounds are skin irritants therefore, a balance

between penetration enhancement and irritation have to be considered. Anionic surfactants can penetrate and interact strongly with the skin. Cationic surfactants have also shown penetration properties but are not used because of their toxic nature.

Examples of commonly used surfactants are:

Anionic surfactants: Dioctylsulphisuccinate, Sodium lauryl sulphate, Decodecyl methylsulphoxide.

Nonionic surfactants: Pluronic F127,Pluronic F68 etc.

Bile salts: Sodium taurocholate, Sodium deoxycholate, Sodium taurolglycocholate.

c. Binary system

These systems apparently open up the heterogeneous multilaminate pathway as well as the continuous pathways.

e.g : Propylene glycol-oleic acid and 1,4-butane diol-linoleic acid.

d. Miscellaneous chemicals

These include urea, a hydrating and keratolytic agent, N,N-dimethyl –m-toluamide, Calciumthioglycolate.

4. **Other excipients**

*Adhesives

* Backing membrane

Adhesives

Adhesive systems should fulfil the following criteria:

* Should not irritate or sensitize the skin or cause an imbalance in the normal skin flora during its contact time with the skin.

* Should adhere to the skin aggressively during the dosing interval without its position being disturbed by activities such as bathing, exercise etc.

* Should be easily removed.

* Should not leave any unwashable residue on the skin.

E.g. Polyacrylates, Polyisobutylene and Silicon based adhesive.

Backing membrane

The primary function is to provide a good bond to the drug reservoir, prevent drug from leaving the dosage forms through the top. It is impermeable substance that protects the product during use on the skin.

e.g: Metallic plastic laminate, Occlusive base plate (aluminium foil), Adhesive foam pad (flexible polyurethane) etc.

APPLICATIONS OF TRANSDERMAL PATCHES[29,30]

1. Transdermal patch of nicotine, which releases nicotine in controlled doses to help with cessation of tobacco smoking.

2. Nitroglycerine patches are sometimes prescribed for the treatment of Angina.

3. Clonidine, the antihypertensive drug and ketoprofen, the non-steroidal anti inflammatory drug are also available in the form of transdermal patches.

4. Transdermal form of the MAOI selegiline, became the first transdermal delivery agent for an antidepressant.

5. Two opioid medications used to provide round-the-clock relief for severe pain are often prescribed in patch form- Fentanyl (marketed as Duragesic) and Buprenorphine (marketed as BuTrans).

REVIEW OF LITERATURE

Meenakshi M *et al*.(2016).,[67] Prepared lamivudine loaded maltodextrin based proniosomes by slurry method using different nonionic surfactants (Span 40, Span 60, Tween 60) in various concentrations. The concentration of cholesterol& maltodextrin were kept constant. The interaction between drug and added exicipients were studied by FT-IR. Further the proniosomes were evaluated for particle size distribution, drug content, entrapment efficiency, *in- vitro* drug release and kinetic studies. The stability study of optimized proniosomes was determined under refrigerated condition ($4^0C \pm 2^0C$) and room temperature ($25^0C \pm 2^0C$). The effect of surfactant concentration on vesicle size, entrapment efficiency and *in-vitro* drug release were studied. The highest entrapment efficiency of 95.02%& cumulative percentage drug release of 93.53% was found for formulation containing maltodextrin,span40 &cholesterol in the ratio 1:3:1.

Sankar *et al*. (2009).,[57] Designed hydrocortisone loaded proniosomes as a novel drug delivery approach for increasing its permeation through the skin. Hydrocortisone proniosomal gel was prepared by coacervation-phase separation method using different combinations of non-ionic surfactants, cholesterol and lecithin. Proniosome formulations were characterized for vesicle size, entrapment efficiency, and drug content uniformity. Span 20: Span 40, Span 20: Span 60 and Span 20: Span 80, combinations showed good entrapment compared with Span: Tween combinations.The results of the study indicated that topical application of hydrocortisone in the form of proniosomes leads to prolonged action.

Goyal *et al*. (2011).,[53] Prepared and evaluated the anti inflammatory activity of gugul- lipid loaded proniosomal gel. The formulated proniosomal based gels were characterized for particle size, entrapment efficiency, *in -vitro* drug release and *in -vivo* anti-inflammatory activity using carrageenan induced rat hind-paw method. The result of the study showed that proniosomal formulation of gugul-lipids holds an immense potential for development of topical herbal anti-inflammatory formulation comparable to topical NSAIDs.

Ankur *et al*. (2007)., [36] Demonstrated a proniosomal carrier system for captopril for the treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a transdermal drug delivery system for captopril was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, lecithin

prepared by coacervation-phase separation method. The formulated systems were characterized for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for 4 weeks. The results of the study showed that proniosomes are a promising prolonged delivery system for captopril and have reasonably good stability characteristics.

Ibrahim *et al*. (2005).,[54] Investigated permeation of ketorolac across excised rabbit skin from various proniosomal gel formulations using franz diffusion cell. Proniosomes prepared with Span 60 provided a higher ketorolac flux across the skin than did those prepared with Tween 20. A change in the cholesterol content did not affect the efficiency of the proniosomes and the reduction in the lecithin content did not significantly decrease the flux ($P>0.05$). The encapsulation efficiency and size of niosomal vesicles formed by proniosome hydration were also characterized by specific HPLC and SEM. The results of the study indicated that proniosome may be a promising carrier for ketorolac and other drugs, especially due to their simple production.

Ammar *et al.* (2011).,[34] Formulated different proniosomal gel bases. They were characterized by light microscopy, and assessed for their drug entrapment efficiency, stability, and their effect on *in- vitro* drug release and *ex- vivo* drug permeation. The lecithin-free proniosomes prepared from Tween 20: cholesterol (9:1) proved to be stable with high entrapment and release efficiencies. The *in- vivo* behaviour of this formula was studied on male rats and compared to that of the oral market product. The result of the study showed that the investigated tenoxicam loaded proniosomal formula proved to be non-irritant, with significantly higher anti inflammatory and analgesic effects compared to that of the oral market tenoxicam tablets.

You Fang *et al.* (2001).,[55] Conducted the skin permeation of estradiol from various proniosomal gel formulations across excised rat skin *in- vitro*. Proniosomes with Span 40 and Span 60 increased the permeation of estradiol across skin. Presence or absence of cholesterol in the lipid bilayers of vesicles did not reveal difference in encapsulation and permeation of the estradiol. He concluded that the types and contents of non-ionic surfactant in proniosome are important factors affecting the efficiency of transdermal estradiol delivery. The results of the study showed that the types and contents of non-ionic surfactant in proniosomes are important factors affecting the efficiency of transdermal estradiol delivery.

Kapil *et al.* (2011)., [56] Fabricated curcumin loaded proniosomes for topical delivery . The drug was encapsulated in the mixture of span80, cholesterol & di-ethyl ether by ether injection method. The prepared formulations were evaluated for size, angle of repose ,drug entrapment, hydration rate & stability under various storage conditions. The in-vitro drug release study was also performed using albino rat skin. Formulation having the Span80: cholesterol ratio of 1:4 showed prolonged release of 61.8% at 24th hr. All the prepared formulations showed entrapment efficiency of 82.3-86.8%.Stability data of the preparation stored under refrigerated condition, room temperature & elevated conditions did not show much variations. Hence they concluded that proniosomes are very stable and promising prolonged delivery system for curcumin.

Kakkar *et al.* (2010).,[58] Prepared non-ionic surfactant vesicles of valsartan by coaservation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and *in vitro* drug release. Stability study was carried out to study the leaching out of the drug from the proniosomal preparation. The encapsulation efficiency of proniosomes prepared with Span 60 was superior to that prepared with Span40. The formulation with 9:2:9 ratio of Span 60, cholesterol and lecithin gave maximum encapsulation efficiency of 71.50% and release rate of 75% over a period of 24 hrs as compared to other formulations.

H Priyanka *et al.* (2015).,[59] Worked on formulation and evaluation of low orally bioavailable drug carvedilol loaded proniosomes as a transdermal drug delivery system. The proniosomal gel was prepared by coacervation phase separation method by using different surfactants like spans 20, 60, 80, tween 20, 80, soya lecithin and cholesterol.The prepared formulations were evaluated for vesicle size analysis, surface morphology studies, encapsulation efficiency, and *in-vitro* drug release. The formulations prepared from span showed smaller vesicle size & high encapsulation efficiency compared to the vesicles prepared from tween. They found out that the vesicles prepared out of span 60 showed highest encapsulation efficiency of 95% & drug release of of 98% over a period of 12 hrs.

Ashish kute *et al.* (2012).,[60] Developed perindopril erbumil loaded proniosomal gel by coaservation phase separation method. Different surfactant ratios were employed for the preparation. Vesicle size, surface morphology, encapsulation efficiency, *in -vitro*, stability study data were evaluated. The size of the vesicles ranged between 15.13µm, encapsulation efficiency between 70.72-76.43%. *In- vitro* release studies showed that as the lipophilicity

increases, the drug release decreases. They showed that the, proniosomal gel with T20:T60 in ratio of 9:1 showed the highest % of drug release (80.03%) over period of 24 hrs.

Ajay *et al.* (2008).,[61] Prepared, characterized and optimized the aceclofenac proniosomes using central composite design and carried out stability studies. Preparation was done using slurry method. Three independent variables selected were molar ratio of drug to lipid (X1), surfactant loading (X2) and volume of hydration (X3). Based on central composite design, 16 formulations were prepared. The prepared formulations were evaluated for percentage drug entrapment & mean volume diameter. Proniosomes with optimum responses were selected using the polynomial equations & contour plots developed using central composite design. Both the proniosomal preparations stored under refrigerated condition & at room temperature were found to be stable.

Tamizharasi S *et al.* (2009).,[62] Reported indomethacin loaded maltodextrin proniosome prepared by slurry method incorporating different ratios of surfactant to cholesterol ratio. The prepared proniosomes were optimized for % drug entrapment. Microscopic studies showed that all the particles have uniform size. The *in -vitro* release studies exhibited prolonged release for a period of 24hrs. The zeta potential values indicated that the proniosomes are stabilized by electrostatic repulsion forces. From stability studies they observed that the formation of niosomes were less at 4ºC, followed by 25ºC &37ºC. They concluded that proniosomes showed maximum entrapment & *in -vivo* evaluation with enhanced bioavailabilty & long lasting drug release profile.

Gamal M *et al.* (2010).,[39] Developed meloxicam loaded proniosomes as a carrier for transdermal delivery. They characterized the proniosomes for entrapment efficiency, surface morphology & *in vitro* drug release. Proniosomes prepared using spans & tweens exhibited high encapsulation efficiency. The prepared proniosomes considerably improved drug penetration & reduced lag time. The formulation prepared from span60 showed greater penetration through skin than those prepared using tween80. The results showed that the proniosomal gel showed better activity than meloxicam gel which showed that these are capable carrier as a transdermal delivery.

Raja K *et al*. (2011).,[63] Fabricated glipizide loaded maltodextrin based proniosome by slurry method with different ratios of surfactant & cholesterol. The formulations were subjected to compatibility studies , angle of repose, SEM, entrapment efficiency, *in vitro* release study & stability study. The formulation having surfactant :cholesterol ratio 3:2, was found to be the

best formulation with highest entrapment efficiency of 84.25% , maximum drug release rate of 99.23% at the end of 24th hr and follows zero order kinetics. The formulation showed appropriate stability when stored under refrigerated condition compared to the reconstituted niosomes.

Anahita Fathi *et al.* (2009).,[64] Established the effect of hydrophilicity & hydrophobicity of surfactants on drug solubility, surface morphology, stability & skin permeation of haloperidol from different formulations. Formulations prepared with single surfactant showed more permeation of the drug than the formulations prepared with 2 surfactants. The number of carbons in the alkyl chain of the non-ionic surfactant affected the *in vitro* permeation of HP through the epidermis and the skin permeation was increased with increase in hydrophilic–lipophilic balance (HLB) value of the surfactant. They concluded that the interfacial tension and surfactant hydrophobicity are useful for elucidating mechanism of skin permeation and for comparing drug fluxes from different proniosomal formulations.

Mohamed *et al.* (2010).,[65] Developed a low dose proniosomal gel containing celecoxib for the treatment of osteoarthritis. All the prepared formulations were subjected to physicochemical evaluations and anti inflammatory studies. The entrapment was greater than 90%. The vesicle shape was determined with the help of transmission electron microscopy. The vesicle size, size distribution, and polydispersity studies were performed using photon correlation spectroscopy. Anti-inflammatory studies were performed using the rat hind-paw oedema induced by carrageenan (1% w/v). The obtained results showed that the proniosomal formulation significantly improved the extent of celecoxib absorption than conventional capsule.

Mokhtar *et al.* (2008).,[66] Fabricated proniosomal gels or solutions of flurbiprofen based on span 20 (Sp 20), span 40 (Sp 40), span 60 (Sp 60), and span 80 (Sp 80) without and with cholesterol. The influence of different processing and formulation variables was demonstrated. The release of the prepared niosomes in phosphate buffer (pH 7.4) was illustrated. Results indicated that the EE% followed the trend Sp 60 (C18) > Sp 40 (C16) > Sp 20 (C12) > Sp 80 (C18).

Pankaj S *et al* .(2013).,[42] Formulated proniosomal gel of clotrimazole using lecithin & cholesterol as encapsulating agents & span and tween of different grades. The gel was evaluated for pH, vesicle size, % encapsulation, drug diffusion study, *ex-vivo* skin permeation and *ex-vivo* drug deposition study &stability study. It was found that formulation containing

span60 showed prolonged release compared to conventional formulation and showed 2 fold increase in drug deposition in the skin compared to conventional cream. Results from antifungal study showed that the developed proniosomal gel is more efficient than the marketed formulation. Stability studies also revealed that the proniosomal gels were stable at 5±3ºC & 25±2ºC. They concluded that the proniosomal gel of clotrimazole could be formulated for sustained release.

Vivane F *et al.* (2012).,[68] Investigated proniosomal carrier systems for acyclovir. Acyclovir proniosomal carrier systems were prepared by three different methods i.e slow spray coating method, coaservation phase separation method & slurry method using different carriers & were subjected to *in-vitro* & *ex-vivo* studies. Characterization of the prepared proniosomes were done by particle size analysis, microscopical examination, IR spectroscopy, DSC*, in-vitro* dissolution and storage studies. Results showed that the prepared vesicles were spherical, discrete with no aggregation. It was observed that in *in -vitro* release studies initially the drug release was faster from proniosomes followed by relatively slower release up to 8 hours. *Ex vivo* intestinal permeability studies showed a faster drug flux from the proniosome formulations than from drug solution and also the formulations were stable at different temperatures. They concluded that the proniosomes are promising carrier for acyclovir.

AIM AND OBJECTIVES

AIM:

The absorption of drugs through the transdermal route improves bioavailability of drugs that might otherwise be metabolized by first-pass during their passage through the gastrointestinal tract. Drug absorption from the transdermal route is mainly via passive diffusion through the lipoidal membrane. Thus, transdermal route of drug delivery has attracted the attention worldwide for optimizing the drug delivery.

Metronidazole is an antifungal agent & can be given through topical route. It has been used in the treatment of various topical as well as systemic diseases which includes: acne, pelvic inflammatory disease, endocarditis, bacterial vaginosis, amoebiasis, trichomoniasis . It has low aqueous solubility, which can affect its dissolution leading to poor BA. Incorporation into proniosomes can lead to reduced dose, improved bioavailability and prolonged release, hence the drug of choice.

The aim of study is to formulate and develop Metronidazole Benzoate loaded proniosomes with different ratios of cholesterol and non-ionic surfactants and incorporation of proniosomes into transdermal patch to achieve prolonged release of drug on topical administration.

OBJECTIVES:

- To prepare proniosomes and evaluate them for angle of repose, drug content, particle size, hydration rate, encapsulation efficiency, *in-vitro* release studies& *ex-vivo* drug permeation studies.
- To incorporate proniosomes into transdermal patch.
- To evaluate proniosomal transdermal patch.

PLAN OF WORK

- Collection of raw materials
- Review of literature
- Calibration curve for Metronidazole benzoate
- Preparation of proniosomes
- Evaluation of proniosome
    1. Angle of repose
    2. Optical microscopy
    3. Drug content analysis
    4. Entrapment efficiency
    5. *In – vitro* drug diffusion study
    6. *Ex-vivo* permeation study
    7. Rate of spontaneity
    8. Vesicle size analysis
    9. Anti- fungal study
    10. Scanning electron microscopy
- Incorporation of proniosomes into transdermal patch.
- Evaluation of proniosomal transdermal patch.
    1. Physical appearance
    2. Thickness
    3. Folding endurance
    4. % Moisture loss
    5. % Moisture absorption

6. Drug content determination

7. Water vapor transmission rate

8. % Flatness

9. In-vitro diffusion study

10. Drug release kinetic data analysis

11. Stability study

DRUG PROFILE

METRONIDAZOLE BENZOATE[22,23,24]

Synonym : Benzoyl metronidazole

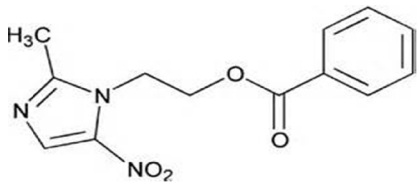
Molecular formula : $C_{13}H_{13}N_3O_4$

IUPAC name :2-(2- methyl-5- nitro-1-H-imidazole-1-yl)ethyl benzoate

Molecular weight : 275.3 g/mol

Description : A white or cream coloured crystalline powder or flakes. 200mg of Metronidazole benzoate is equivalent to 125mg of Metronidazole.

Structure of Metronidazole Benzoate:



Category : Antiamoebic, antiprotozoal , antibacterial

Solubility : Practically insoluble in water, freely soluble in methylene chloride, soluble in ethanol.

Route of administration : Oral, rectal, vaginal, topical.

Dose : Amoebiasis- Equivalent of 400mg of metronidazole thrice daily for 5-10 days. Anaerobic infections: 500mg every 6 hrs.

Half-life : 7-8hrs

Melting point : 98-102ºC

Uses : Pelvic inflammatory disease, endocarditis, bacterial vaginosis, amoebiasis, trichomoniasis.

Mechanism of action : It is a bactericidal, amoebicidal & trichomonicidal drug. It is reduced by low redox potential electron transfer proteins (nitroreductases- ferredoxin) to unidentified polar products

which lack the nitrogroup. These reduction products inhibit nucleic acid synthesis by disrupting the DNA of microbial cells.

Pharmacokinetic profile : Oral B.A = 60%

PPB = 20%

V_d = 0.510- 1.10L/Kg

ADR **:** More serious – Breathing difficulty, swelling of lips,tongue,face or throat

Less serious – Mild burning or stinging when applied, numbness or tingly feeling in hand & feet cough , stuffy nose, sore throat & cold vaginal itching

Storage **:** Store below 25ºC, protected from light.

EXCIPIENT PROFILE

CHOLESTEROL[13]

Synonym :Cholesterin, Cholesterolum

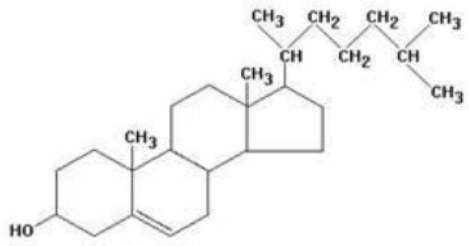
Chemical formula : $C_{27}H_{46}O$

Molecular weight : 386.6535g/mol

Chemical name : Cholest-5-en-3ß-ol

Description : Cholesterol occurs as white or faintly yellow, odourless, pearly leaflets, needles, powder or granules. On prolonged exposure to light & air, it acquires a yellow to tan colour.

Structure of cholesterol:



Functional category : Emollient, emulsifying agent

Melting point : 148.5ºC

Boiling point : 360ºC

Water solubility : 00.095mg/L (at 30ºC)

Applications : It is used in cosmetics & topical pharmaceutical formulations as an emulsifying agent. It imparts water absorbing power to ointments. It is the major sterol of higher animals, & is found in all body tissues, especially in brain &spinal cord.

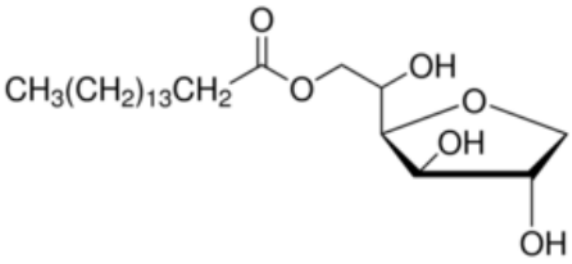
SPAN 40[13]

Synonym : Sorbitan monopalmitate

Chemical formula : $C_{22}H_{42}O_6$

Molecular weight : 402.57g /mol

Structure of span 40 :



Properties of span 40

Related categories : Biochemical reagents, cleaner, detergent

Description : Non-ionic

Melting point : 46-47ºC

HLB value : 6.7±10

SPAN 60[14]

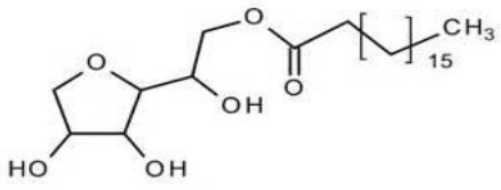
Synonym : Sorbitane monostearate, sorbitan stearate

Chemical name : Span60

Chemical formula : $C_{24}H_{46}O_6$

Description : It is an ester of sorbitan & stearic acid & is sometimes referred to as synthetic wax. It is an non-ionic surfactant with emulsifying, wetting & dispersing properties.

Structure of span 60:



Related categories : Biochemical reagent, cleaner, detergent

HLB value : 4.7

Applications : Span 60 has been used in a study to assess encapsulation of doxorubicin in niosomes as a route to tumour targeting. It has been also been employed to investigate the use of non-ionic surfactants as contrast agents for the use in diagnostic ultrasound.

TWEEN60[15]

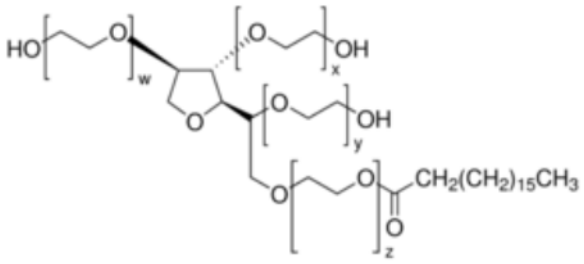
Synonym : Polysorbate monostearate

Chemical name : Tween60

Chemical formula : $C_{64}H_{42}O_{26}$

Description : It is a yellow oily liquid with characteristic odour, soluble in ethanol, insoluble in mineral oil & vegetable oil.

Structure of tween 60:



Molecular weight : 1312g/mol

Saponification value : 45-55

Acid value : ≤2

Specific gravity : ≈1.10

Viscosity : ≈400mPas

Flash point : 149ºC

HLB value : 14.9

Moisture content : 3%

Surface tension : 42.5cps

Applications : Emulsifying agent, non-ionic surfactant, solubilising agent, wetting agent, suspending agent.

TWEEN20[69]

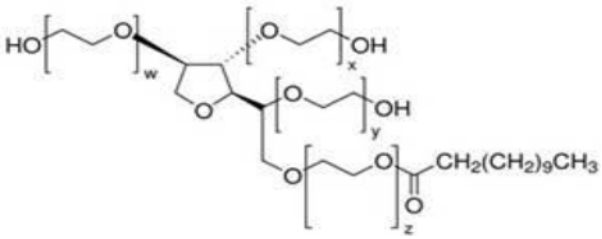
Synonym : Polysorbate20, PEG (20) sorbitan monolaurate, Alkest TW20

Chemical name : Polyoxyethylene (20) sorbitan monolaurate

Chemical formula : $C_{58}H_{114}O_{26}$

Description : Clear yellow to yellowish green viscous liquid

Structure of tween 20:



Molecular weight: 1227.54g/mol

Density : 1.1g/mL

Boiling point : >100ºC

HLB value : 16.7

Applications : * It has been widely used as an excipient in pharmaceutical applications to stabilise emulsions &suspensions.

*As a wetting agent in flavoured syrups.

*As a washing agent in immunoassays.

* As a solubilising agent of membrane proteins.

*To stabilise purified protein derivative solution used in skin testing for tuberculosis exposure.

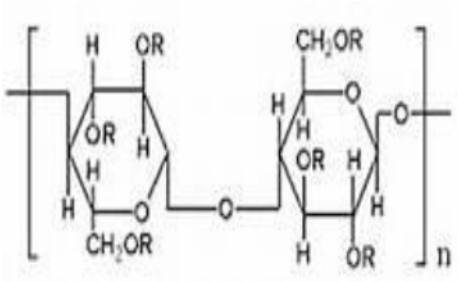
HYDROXY PROPYL METHYL CELLULOSE[70]

Synonym : Benecel MHPC, Hypromellosum, Methocel, Pharmacoat

Chemical formula : Cellulose hydroxypropylmethyl ether

Description : It is an odourless, tasteless, white or creamy white fibrous or granular powder

Structure of HPMC :



Where R is H,CH_3 or $CH_3CH(OH)CH_2$

pH : 5.5-8.0 for 1% aqueous solution

Melting point : 190-208ºC

Solubility : Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95%), & ether, but soluble in mixtures of ethanol & dichloromethane, methanol& dichloromethane & mixtures of water & alcohol.

Applications : It is widely used in oral, ophthalmic, & topical formulations. In oral products, it is primarily used as tablet binder, in film coating, as matrix for extended release tablet formulations.

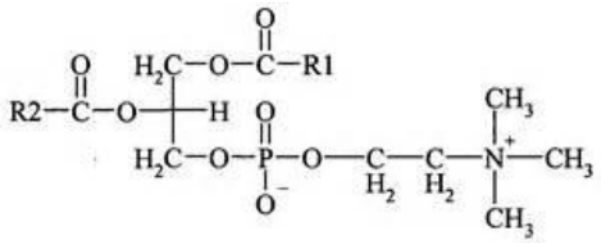
LECITHIN[18]

Synonym : Lecithol,Vitellin, Kelecin, Granulestin

Chemical name : 1,2- diacyl-sn-glycero-3-phosphocholine

Description : Vary in colour from brown to light yellow

Structure of lecithin:



Functional category : Emollient, emulsifying agent, solubilising agent

Solubility : Soluble in aliphatic & aromatic hydrocarbons, halogenated hydrocarbons & mineral oil.

Density : 0.5g/cm^3

Isoelectric point : 3.5

Compatibility : Incompatible with esterase owing to hydrolysis.

Stability : Lecithin decomposes at extreme pH

Moisture content : Hygroscopic in nature

Specific gravity : 0.97g/cm^3

Safety : Non-toxic, excessive consumption may be harmful.

Applications : Lecithin is used in wide variety of pharmaceutical preparations like aerosol inhalations, i.m injections & oral suspensions. It is mainly used as dispersing, emulsifying & stabilising agent.

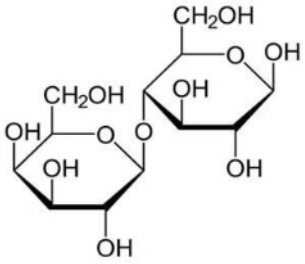
LACTOSE[16]

Synonym : Fast flow, Lactochem, Tablettose, Zeparox, Granulac, Microfine, Pharmatose.

Molecular weight : 342.30g/mol

Description : It is white to off white crystalline powder. It is odourless & slightly sweet in taste.

Structure of lactose:



Functional category : Tablet & capsule diluent

Solubility : It is practically insoluble in chloroform, ethanol, ether & partially soluble in water.

Loss on drying : Less than 1.0% for monohydrate & 0.5% for anhydrate forms.

Storage & Stability : Under humid conditions above 80% mould may grow. It develops brown colour on storage. It should be stored in well closed container in a cool &dry place.

Applications : Lactose is widely used as filler or diluents in tablets & capsules. It is also used as a carrier for inhalational & lyophilized products.

MANNITOL[17]

Synonym : Manita, Manitol, Manna sugar, Mannit, D Mannite, Mannite, Mannitolum

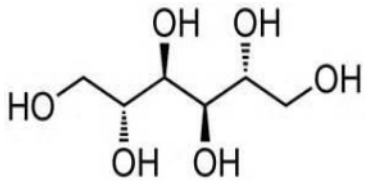
Chemical name : D mannitol

Molecular weight: 182.17g/mol

Description : It occurs as a white, odourless, crystalline powder or free flowing granules. It has a sweet taste & imparts a cooling sensation in the mouth. It shows polymorphism.

Chemical formula : $C_6H_{14}O_6$

Structure of mannitol:



Functional category: Lyophilisation & plasticizing agent, sweetening agent, tablet & capsule diluents, tonicity agent.

Stability & Storage : Mannitol is stable in dry state & in aqueous solution. It should be stored in cool, dry place.

Applications : In pharmaceutical preparations it is used as a diluent. It has been used to prevent thickening in aqueous antacid suspensions, as a plasticizer in soft gelatine capsules, as a carrier in dry powder inhalers & also in lyophilised preparations to improve its appearance.

MATERIALS AND METHODS

Table 1: List of materials used

Materials	Supplier/ Manufacturer
Span40	Lobachemie pvt. Ltd, Mumbai
Metronidazole benzoate	Sanofi Aventis, Goa
Span60	Lobachemie pvt. Ltd, Mumbai
Tween60	Lobachemie pvt. Ltd, Mumbai
Tween20	Lobachemie pvt. Ltd, Mumbai
Lactose	SD Fine Chemicals, Mumbai
Mannitol	SD Fine Chemicals, Mumbai
Cholesterol	Lobachemie pvt . Ltd, Mumbai
Lecithin	Hi-Media laboratories pvt. Ltd, Mumbai
HPMC	Lobachemie pvt . Ltd, Mumbai
Chloroform	Hi-Media laboratories pvt. Ltd, Mumbai
Ethanol	Finar Chemicals Ltd, Mumbai

Table 2: List of instruments used

Instruments	Supplier/ Manufacturer
Single pan analytical balance	Amandi, Mumbai
Hot air oven	Technico, Mumbai
Magnetic stirrer	Remi, Mumbai
Sonicator	Sonics, USA
Dessicator	Sorbead, Mumbai
Humidity chamber	Technico, Mumbai
Franz diffusion cell	Self fabricated, Coimbatore
UV spectrophotometer	Schimadzu, Schimadzu corporation, Philippines
FT/IR spectrometer 4100	Jasco, Johannesburg, South Africa
Rotary vacuum evaporator	Superfit, India
Stability chamber	Technico, Chennai, India

METHODOLOGY

Determination of λ_{max} of Metronidazole Benzoate:

The drug solution of 10µg/ml was prepared in ethanol. The solution was sonicated for 10 mins. This drug solution was scanned in the UV region of 200-400nm & the overlaid spectra was recorded. From the spectra, the λ_{max} was found to be 309nm.

Determination of Standard curve

Weighed accurately 50mg of Metronidazole Benzoate and dissolved in 2.5ml of ethanol . This was made up to 50ml with distilled water in a standard flask & sonicated for 3mins. Then the solution was serially diluted to get concentrations of 10, 20,30,40,50 µg/ml. The absorbance of the solutions were measured in UV spectrophotometer at 309nm. The calibration curve was plotted by taking concentration of the solution in µg/ml on X-axis & absorbance on Y-axis & regression co-efficient R^2 was calculated.

Drug excipient compatibility studies

IR spectra matching approach was done for detecting any possible chemical interactions between the drug& the excipient. A physical mixture of the drug & the excipient (1:1) was prepared & mixed with suitable quantity of KBr. About 100mg of mixture was compressed to form a transparent pellet using a hydraulic press at6 tons pressure. It was scanned from 4000-400cm^{-1} in FTIR spectrometer. The IR spectrum of the physical mixture was compared with the IR spectrum of the pure drug & matching was done to detect any appearance or disappearance of peaks.

Surface morphology of proniosome using SEM[32]

To detect the surface morphology of the proniosome , SEM of the complex was performed using Scanning Electron Microscope at STIC, Cochin University, Ernakulam . Double sided carbon tape was affixed on aluminum stubs. The powder sample of proniosomes was sprinkled onto the tape. The aluminum stubs were placed in the vacuum chamber of a scanning electron microscope. The sample was observed for morphological characterization using secondary electron detector attached to scanning electron microscopy.

FORMULATION OF PRONIOSOMES

The proniosomes were prepared using slurry method. The carrier was taken in RBF and the entire volume of surfactant dissolved in the organic solvent was added to form a slurry. The flask was then attached to the rotary evaporator & vacuum was applied until a dry free flowing powder was obtained. The flask was removed from the evaporator and kept under vacuum overnight. The obtained proniosomal powder was stored in sealed container at4ºC.

Table3: Formulation table of proniosomes

Formulation code	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}
Drug(mg)	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Cholesterol(mg)	1000	1000	1000 lecithin	1000	1000	1000	1000	1000	1000	250	500	1000	1000	1000
Span40(mg)	1000	2000												
Span60(mg)			1000	2000					250	1000	1000	1000	500	750
Tween20(mg)					1000	2000								
Tween60(mg)							1000	2000						
Lactose(mg)									1000					
Mannitol(mg)	1000	1000	1000	1000	1000	1000	1000	1000		1000	1000	1000	1000	1000
Chloroform(ml)	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Ethanol(ml)	5	5	5	5	5	5	5	5	5	5	5	5	5	5

EVALUATION OF PRONISOMES

Angle of repose[33]

The angle of repose of dry proniosomal power was measured by funnel method. The proniosome powder was poured into a funnel which was fixed at a position so that the orifice of the funnel is 2cm above the level surface. The powder flows from the funnel to form a cone on the surface & the angle of repose was then calculated by measuring the height of the cone & the diameter of the base. The angle of repose was then calculated from the following formula:

$$\tan\theta = h/r \qquad (3)$$

Where,

θ= angle of repose

h= height of the cone

r = radius of the cone base

Table 4: Standard values for angle of repose

Angle of repose (°)	Type of flow
<25	Excellent
25-30	Good
30-40	Satisfactory
>40	Poor

Optical microscopy[34]

It is done to confirm the vesicle formation. A thin layer of proniosomal powder was placed on cavity slide. A drop of water was added through the sides of the coverslip into the cavity silde while under microscope & observed.

Drug content analysis[35]

Proniosomes equivalent to 30mg of drug was taken in a standard volumetric flask. They were lysed with 10ml ethanol by shaking for 15min. Diluted to 100ml with distilled water & sonicated for 3min. Aliquots were withdrawn , absorbance was measured spectrophotometrically at 309nm & the drug content was calculated from the calibration curve.

Entrapment efficiency[36]

100mg of proniosome was dispersed in little warm water to allow the formation of niosomes. Then added 5% ethanol upto 10ml & sonicated for 10 min. Then the dispersion was centrifuged at1800 rpm for 40 min at 5ºC . The clear fraction was diluted appropriately with distilled water & measured the absorbance at 309nm. The % encapsulation was calculated from the equation.

% Encapsulation efficiency = (Total drug- Free drug / Total drug) ×100 (4)

***In-vitro* drug diffusion study through egg membrane**

Preparation of egg membrane[37]:

From local departmental store egg was purchased. The skin was removed carefully from the outer region of the egg and separated from the underlying membrane. The outer skin of egg was removed with the help of 0.1 N HCl with constant stirring. After separating the full membrane, the membrane was washed with using phosphate buffer pH 7.4. The membrane was now used for further experimental work.

Drug diffusion through egg membrane[38]:

The *in-vitro* diffusion study was done using Franz Diffusion Cell. The capacity of the receptor compartment is 15ml. The egg membrane was mounted between the donor & the receptor compartments. Weighed amount of proniosomal powder (100mg) was placed in the donor compartment &the receptor compartment was filled with 5%ethanol . The receptor fluid was stirred continuously by a magnetic stirrer. At each sampling interval , samples were withdrawn for a period of 8hrs & were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analysed spectrophotometrically at 309nm.

***Ex-vivo* skin permeation study**[39,40](optimised batch)

The permeation of Metronidazole Benzoate from proniosomal powder was investigated by using *in-vitro* Franz Diffusion Cell. The abdominal chicken skin was obtained from slaughter house & adhering subcutaneous fat was carefully cleaned. To remove the extraneous debris &leachable enzymes, the dermal side of the skin was kept in contact with physiological saline solution for 1hr before starting the permeation experiment.

The skin was mounted on the receptor compartment with the stratum corneum facing towards the donor compartment. The receptor compartment was filled with 15ml of 5% ethanol maintained at 37ºC & was constantly stirred using a magnetic stirrer. 1g of proniosomal powder was placed on the skin in the donor compartment. . At each sampling interval , samples were withdrawn for a period of 12hrs & were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analysed spectrophotometrically at 309nm.

Figure 3: Abdominal skin of chicken

Rate of spontaneity(hydration)[41]

10 mg of proniosomal powder (optimized batch) was transferred to a test tube & spread uniformily . 1 ml of 0.9% saline solution was added along the sides of the walls & kept aside without agitation . After 15 min a drop was withdrawn &placed on Neubars chamber & the no:of niosomes eluted from proniosomes were counted.

Vesicle size analysis[35] (optimized batch)

Hydrated proniosomal powder was observed under optical microscope at 100X magnification. The sizes of 100-200 vesicles were measured using calibrated stage & eye – piece micrometer fitted in optical microscopy.

Anti-fungal study[42]

Anti –fungal study was carried out to ascertain the biological activity of the optimized formulation& compared with the standard drug(pure Metronidazole) against *Candida albicans.*

A layer of Sabouraud dextrose agar media(20ml) was seeded with 0.2ml of the test micro-organism & allowed to solidify in the petri-plate. Cups were made with sterile borer at4mm diameter on the solidified agar layer. 50 mg of the optimized formulation was taken &suspended in normal saline. The 12thhr release sample solution was poured into the cup for microbial assay. After keeping the petri-plates at room temperature for 4hr, the plates were incubated at 37ºC for 24 hrs. The diameter of zone of inhibition was measured.

FORMULATION OF TRANSDERMAL PATCHES[43]

The optimized batch of proniosome was loaded to the transdermal patch prepared by mixing 200mg HPMC & 2% propylene glycol in solvent chloroform:ethanol(2:1) & poured into a

petri plate . The rate of evaporation of solvent was controlled by placing an inverted funnel over the petri plate . After 24 hrs the dried film was take out .

EVALUATION PARAMETERS OF PATCH

Physical appearance

All the transdermal films were visually inspected for colour, clarity, flexibility and smoothness.

Thickness uniformity[44]

The thickness of the formulated film was measured at 5 different points using a Vernier calliper and average thickness was calculated.

Folding endurance[45]

The folding endurance was measured manually for the prepared films. A strip of film 1cm^2 was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking or cracking gives the value of folding endurance.

Percentage moisture absorption[46]

The films were weighed accurately and placed in a desiccator containing 100 ml of saturated solution of potassium chloride. After 3 days, the films were taken out and weighed again. The percentage moisture absorption was calculated using the formula:

$$\% \text{ Moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad (5)$$

Percentage moisture loss[47]

The films were weighed accurately and placed in a desiccator containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed again. The percentage moisture loss was calculated using the formula:

$$\% \text{ Moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad (6)$$

Drug content determination[48]

A specified area of patch(1cm∗1cm) was dissolved in ethanol& made up the volume to 100ml with distilled water. Then the whole solution was ultrasonicated for 15min. After filtration, the drug was estimated spectrophotometrically at wavelength of 309nm and determined the drug content.

Water vapour transmission rate[37]

Glass vial of equal diameter were used as transmission cells. The transmission cell was washed thoroughly and dried in an oven. The prepared film was fixed over the edge of the glass vial containing 3 gm of fused calcium chloride as a desiccant by using an adhesive. Then the vial was placed in a desiccator containing saturated solution of potassium chloride. The vial was taken out periodically and weighed for a period of 72 h. The water vapour transmission rate was calculated using the formula :

$$WVT = WL/S \qquad (7)$$

Where,

W: Water vapor transmitting in g

L: Thickness of patch in cm

S: Exposed surface area in cm

Percentage flatness test[49]

This test is done to ensure that the patches do not constrict with time. Three longitudinal strips were cut out from each film: 1 from the center, 1 from the left side and 1 from the right side. The length of each strip was measured and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness . The percentage constriction can be calculated using the formula:

$$\% \text{ Constriction} = I1\text{-}I2/\ I1 \times 100 \qquad (8)$$

Where,

I1: initial length of each strip

I2: Final length of each strip

***In-vitro* diffusion study**[50]

The *in-vitro* diffusion study was done using Franz Diffusion Cell. The capacity of the receptor compartment is 15ml. The egg membrane was mounted between the donor & the receptor compartments. The formulated patch was cut into size of $2cm^2$ & was placed above the egg membrane in the donor compartment . The receptor compartment was filled with 5%ethanol. The receptor fluid was stirred continuously by a magnetic stirrer. At each sampling interval, samples were withdrawn for a period of 12hrs & were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analysed spectrophotometrically at 309nm.

Drug release kinetics[51]

To know the release kinetics , the data obtained from the *in-vitro* release profile was fitted into various models like

* Zero order kinetic model: cumulative percent drug release v/s time

* First order kinetic model: log cumulative percent drug remaining v/s time

* Higuchi's model: cumulative percent drug release v/s square root of time

*Korsmeyer- Peppas model : log cumulative percent drug release v/s log time

Zero order kinetics:

It describes the system in which the drug release rate is independent of its concentration.

$$\mathbf{Q_t = Q_o + K_o t} \qquad \mathbf{(9)}$$

Where,

Q_t= Amount of drug dissolved in time t

Q_o= Initial amount of drug in the solution , which is often 0

K_o=Zero order release constant

If the release pattern obeys zero order, then the plot of Q_t v/s t will give a straight line with a slope of K_o & an intercept at 0.

It is used to describe the drug dissolution of different types of modified release pharmaceutical dosage forms, as in case of some transdermal systems. The drug release from the dosage form is independent of the amount of drug remaining in the system.

First order kinetics

It describes the drug release from the systems in which the release rate is concentration dependent.

$$\log Q_t = \log Q_o + kt/2.303 \qquad (10)$$

Where,

Q_t= Amount of drug released in time t

Q_o= Initial amount of drug in the solution

k= First order release constant

If the release pattern obeys first order, then the plot of $\log(Q_o-Q_t)$ v/s t will be straight line with a slope of kt/2.303 & an intercept at t= $\log Q_o$.

This type of dissolution profile is shown by pharmaceutical dosage forms containing water soluble drugs in porous matrices. The release of the drug is proportional to the drug remaining in its interior portion.

Higuchi model

According to this model, the fraction of drug from a matrix is proportional to the square root of time.

$$M_t/M_\alpha = kHt^{1/2} \qquad (11)$$

Where,

M_t & M_α = Cumulative amounts of drug release at time t & at infinity

kH = Higuchi dissolution constant(reflects formulation characteristics)

If the Higuchi model of drug release (i.e Fickian diffusion) is obeyed, then the plot of M_t/M_α v/s $t^{½}$ will be straight line with slope kH.

Korsmeyer – Peppas model (Power law)

The power law describes the drug release from the polymeric system in which the release deviates from Fickian diffusion. It is expressed using the following equation;

$$M_t/M_\alpha = ktn \quad (12)$$

$$\log[M_t/M_\alpha] = \log k + n \log t \quad (13)$$

Where,

M_t & M_α= Cumulative amounts of drug released at time t & at infinity

k =Constant incorporating structural & geometrical characteristics of CR device

n =Diffusional release exponent indicative of the mechanism of drug release for drug dissolution

To characterize the release mechanism , the dissolution data $\{M_t/M_\alpha < 0.6\}$ are evaluated.

A plot of $\{M_t/M_\alpha\}$ v/s log t will be linear with slope n & intercept value of log k. Antilog of k gives the value of k. Peppas used the n value in order to characterize different release mechanisms as shown below:

Table 5: Release mechanisms

'n' value	**Drug release**
<0.5	Fickian
0.5<n<1	Non - Fickian
>1	Case II transport

Stability studies[52]

The best formulation was tested for its stability. Stability studies were conducted according to the ICH guidelines, by storing the patch at accelerated temperature 40±2 °C and75± 5% RH for 45 days. The samples were withdrawn initially, 30th & 45th day and analyzed suitably for the physical characteristics, drug content and drug release.

RESULTS AND DISCUSSION

Determination of λ_{max} of Metronidazole benzoate by UV spectrophotometer

The standard stock solution of Metronidazole benzoate was prepared in ethanol and scanned by UV spectrophotometer between 200-400nm. The UV absorption spectrum of Metronidazole benzoate showed λ_{max} at 309nm & the same was used as analytical wavelength for further analysis.

Calibration curve of Metronidazole benzoate

The calibration curve for Metronidazole benzoate was developed in 5% ethanol at wavelength of 309nm using UV spectrophotometer. The linearity of the curve was found to be in the range of 10-50µg/ml. The regression coefficient value was found to be 0.9977.

Table 6: Calibration curve of Metronidazole benzoate

Concentration (µg/ml)	Absorbance (nm)
10	0.0709
20	0.3145
30	0.612
40	0.8604
50	1.1862

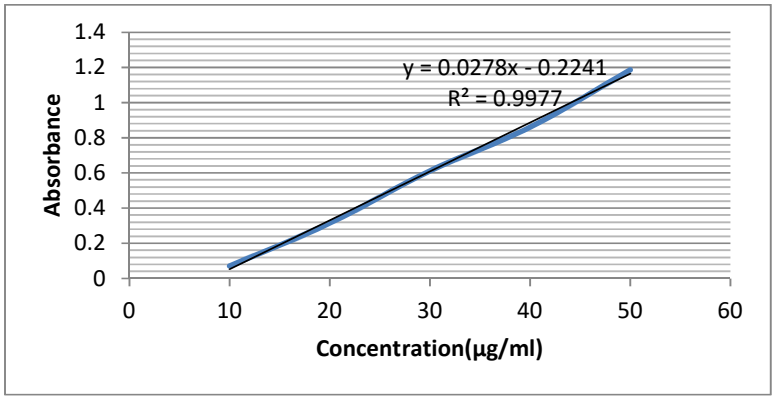


Figure4: Standard graph of Metronidazole benzoate

Drug polymer compatibility studies

The IR spectra of drug and the drug with other excipients are shown. The FT-IR study showed that there is no major change in the position of peak obtained in drug alone and in the mixture of drug with excipients. Hence it can be confirmed that there are no major interactions between the drug & the excipients.

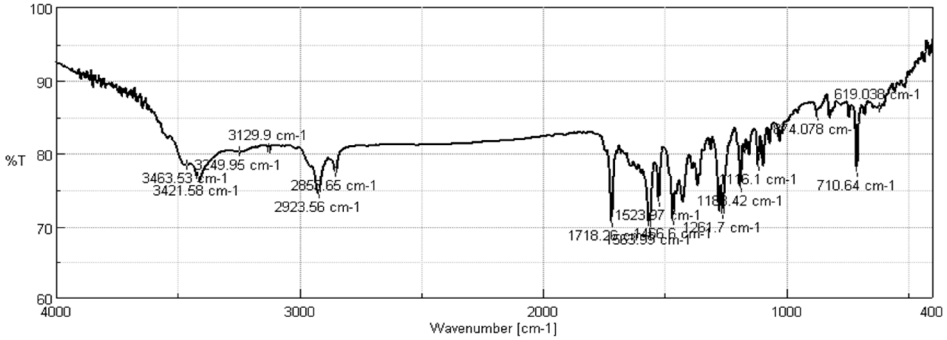


Figure5: FT-IR spectrum of Metronidazole benzoate pure drug

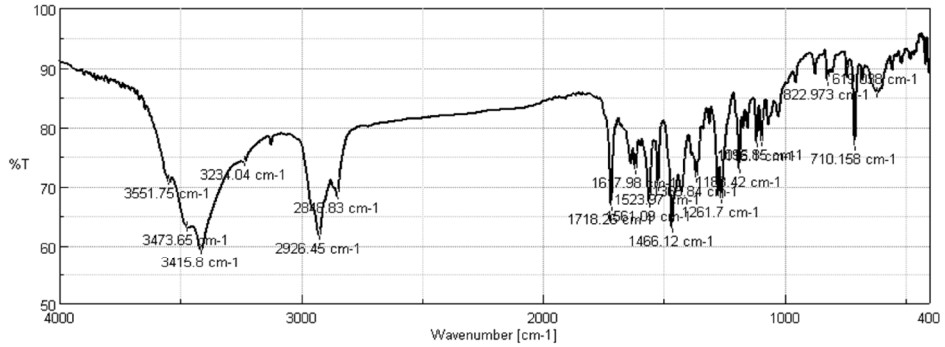


Figure6: FT-IR spectrum of Metronidazole benzoate pure drug+ Cholesterol

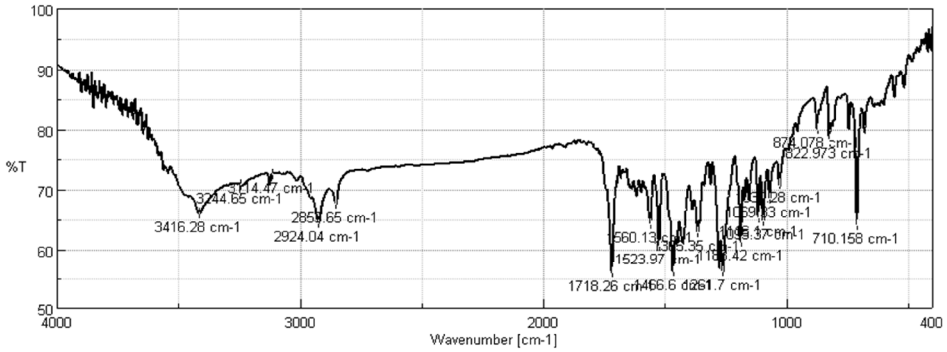


Figure7: FT-IR spectrum of Metronidazole benzoate pure drug+ Lecithin

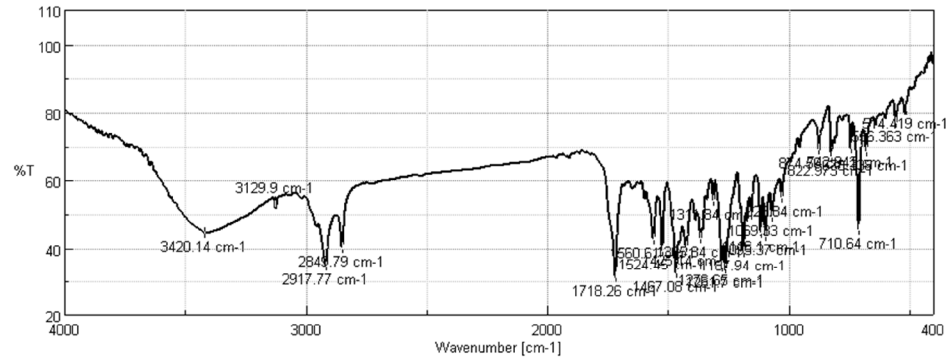


Figure8: FT-IR spectrum of Metronidazole benzoate pure drug+ Span40

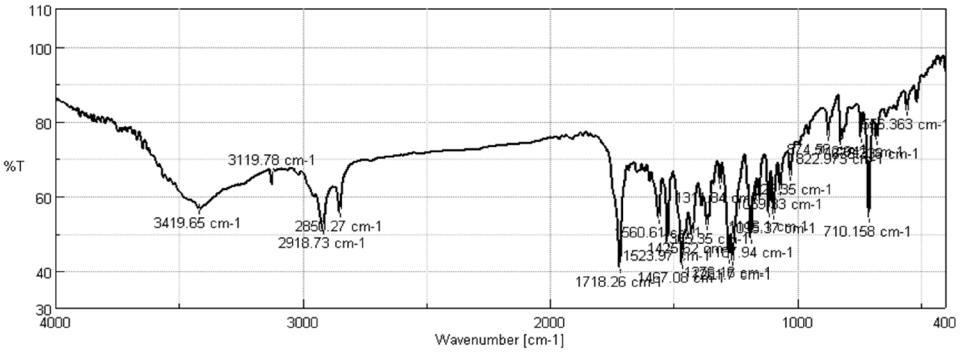


Figure 9: FT-IR spectrum of Metronidazole benzoate pure drug+ Span60

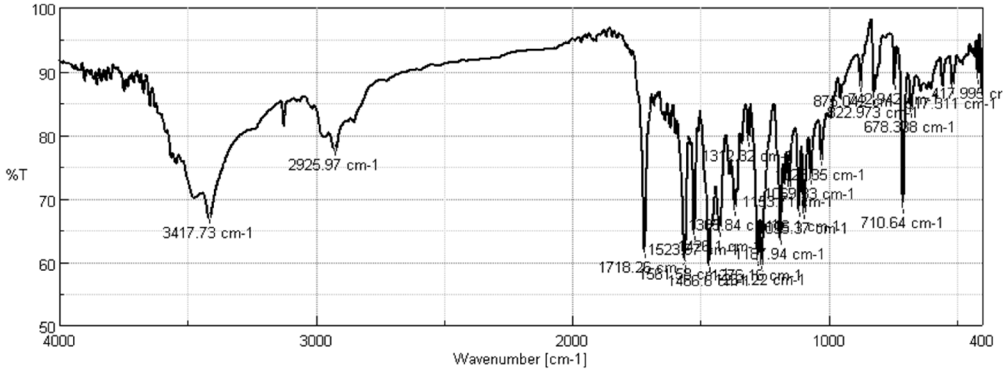


Figure10: FT-IR spectrum of Metronidazole benzoate pure drug+ HPMC

Table7: Interpretation of FTIR spectra of drug and excipients

Drug & excipients	Functional group/ wave number(cm^{-1})					
	Mono - substituted benzene	C=O stretching	N-O stretching in aromatic ring	C=O stretching	Sp^2 CH stretching	C-N stretching
Metronidazole benzoate	710.64	1718.26	1523.97	1261.7	3421.58	1466.6
MB+ Cholesterol	710.158	1718.26	1532.97	1261.7	3415.8	1466.12
MB+ Lecithin	710.158	1718.26	1523.97	1261.7	3416.28	1466.6
MB+Span40	710.64	1718.26	1524.45	1266.65	3420.14	1467.08
MB+Span60	710.158	1718.26	1523.97	1266.16	3419.65	1467.08
MB+ HPMC	710.64	1718.26	1523.97	1261.22	3417.73	1466.6

EVALUATION OF PRONIOSOMES

Angle of repose

Table8: Results of angle of repose

Formulation code	Angle of repose(º)
F_1	21.9
F_2	28.06
F_3	35.2
F_4	23.49
F_5	21.14
F_6	23.49
F_7	23.9
F_8	23.06
F_9	25
F_{10}	24.3
F_{11}	24.15
F_{12}	21.14
F_{13}	22.6
F_{14}	23.06

From the above table, it was observed that all the formulations have good to excellent flow character. F_{12} formulation showed the best flow property.

Optical microscopy

Optical microscopy was performed by viewing the formulations under microcope. It was observed that all the preparations showed vesicle formation. The vesicles formed were found to be of uniform size & shape.

Drug content analysis

Table 9: Results of drug content studies

Formulation code	Drug content(%w/w)
F_1	32.15
F_2	23.11
F_3	43.17
F_4	34.67
F_5	47.53
F_6	51.15
F_7	29.95
F_8	56.66
F_9	37.62
F_{10}	39.19
F_{11}	22.9
F_{12}	75.36
F_{13}	19.83
F_{14}	29.76

From the drug content results, it was observed that formulations showed drug content in the range of 19.8-75.4%w/w. It was also observed that as the concentration of the sorbitan surfactants were increased, showed a decrease in the drug content. Conversely an increase in the concentration of the tween, gave increased drug content. The cholesterol content also influenced the amount of the drug present. Increasing the cholesterol content gave a rise in the drug concentration. This may be due to the lipophilic nature of the drug. In F_3 cholesterol was replaced by lecithin, but it showed less drug content. The formulation F_{12} showed maximum drug content.

Entrapment efficiency

Formulation code	Percentage entrapment efficiency
F_1	86.94
F_2	77.09
F_3	89.2
F_4	72.8
F_5	50.92
F_6	45.85
F_7	50.63
F_8	65.87
F_9	70.6
F_{10}	83
F_{11}	88.6
F_{12}	89.85
F_{13}	85.2
F_{14}	88.5

Table 10: Results of entrapment efficiency

The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The formulations showed entrapment efficiency in the range of 45.85-89.85%. Formulations made from span showed more encapsulation of the drug than the one made from tween. In F_{10} & F_{11} , the entrapment efficiency of F_{11} was more due to increased cholesterol content as low cholesterol content gives rise to smaller vesicles hence reducing the E.E. From all the preparations , the formulation containing span 60: cholesterol ratio 1:1 (F_{12})showed highest entrapment of the drug.

***In –vitro* drug diffusion study**

Table11: Results of drug release studies

Time (hrs)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}
0.5	17.73	18.39	12.04	14.10	9.66	13.49	17.82	7.68	13.20	12.11	17.67	7.20	22.02	19.40
1	28.87	24.56	16.61	19.40	13.11	18.41	25.00	10.40	19.85	18.21	24.26	15.45	29.00	27.49
2	30.70	24.90	16.96	20.71	15.21	22.96	26.55	14.60	22.67	21.69	24.97	28.71	31.48	29.33
3	32.81	37.44	19.90	21.76	17.02	25.41	28.15	19.30	27.65	27.92	30.06	36.74	35.36	30.98
4	37.28	42.08	21.06	27.35	19.27	28.10	32.16	21.90	36.73	34.33	33.02	47.24	38.11	33.65
5	42.02	43.32	22.67	32.28	24.63	31.82	36.24	23.42	40.22	37.59	41.55	54.29	41.27	38.85
6	45.47	44.99	26.73	35.00	29.80	34.15	42.18	28.05	47.95	41.54	47.19	64.36	48.19	42.39
7	50.29	48.92	28.07	39.83	35.13	39.57	46.86	30.40	53.07	46.71	52.88	74.22	57.96	45.54
8	54.60	55.71	32.44	46.80	36.64	44.38	55.41	36.77	58.36	53.26	57.6	82.05	61.43	49.60

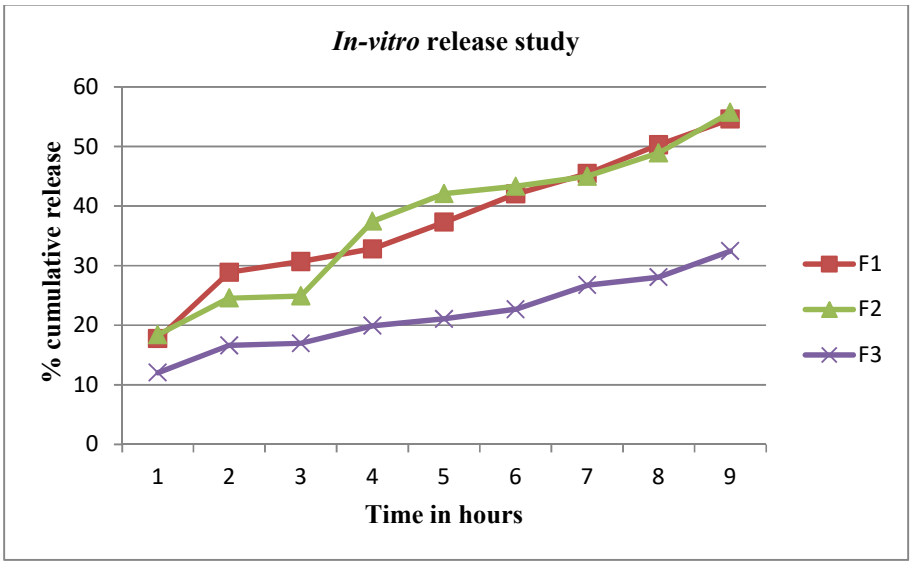


Figure11:*In-vitro* comparative diffusion profile of proniosomal formulations containing Metronidazole Benzoate(F_1-F_3)

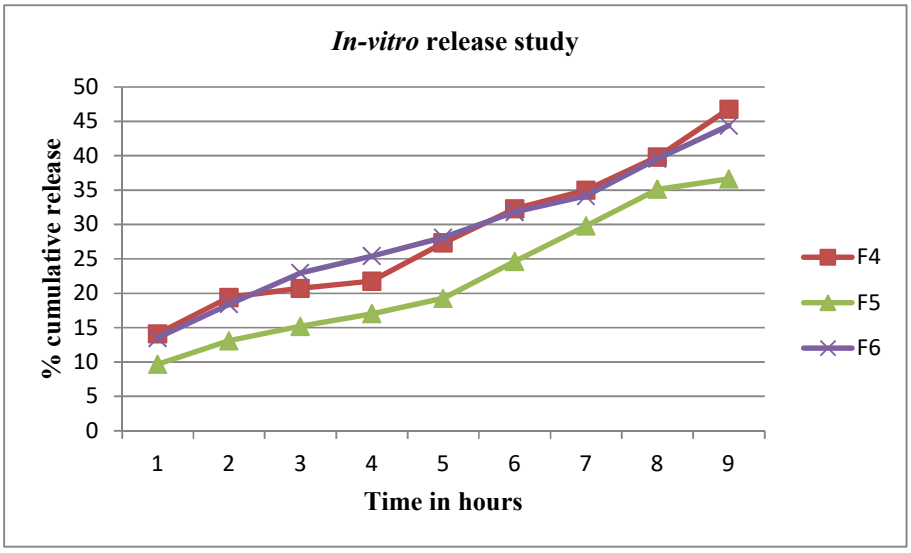


Fig12:*In-vitro* comparative diffusion profile of proniosomal formulations containing Metronidazole Benzoate(F_4-F_6)

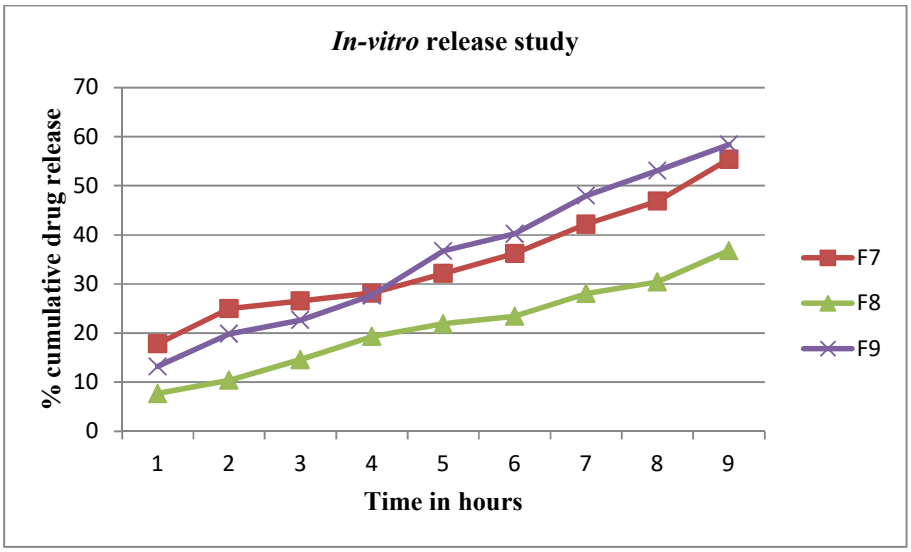


Figure13:*In-vitro* comparative diffusion profile of proniosomal formulations containing Metronidazole Benzoate(F_7-F_9)

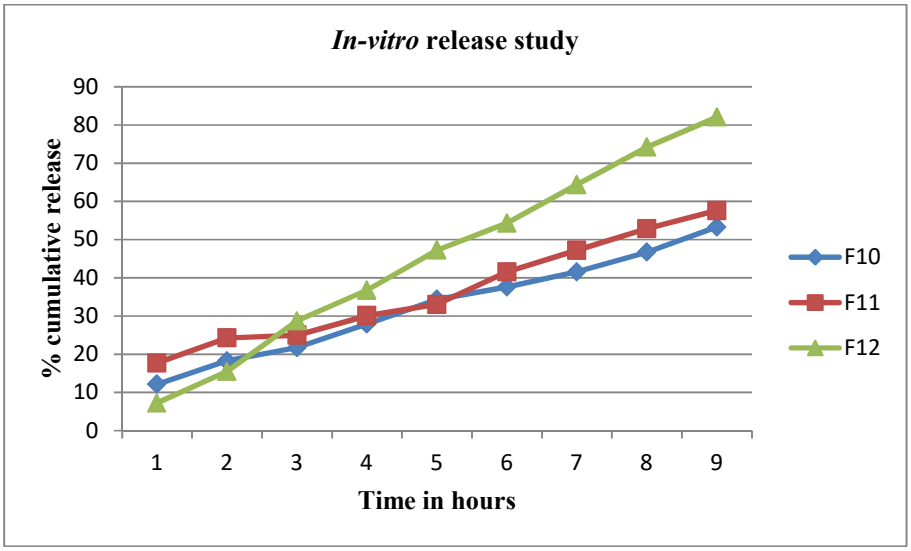


Figure14:*In-vitro* comparative diffusion profile of proniosomal formulations containing Metronidazole Benzoate(F_{10}-F_{12})

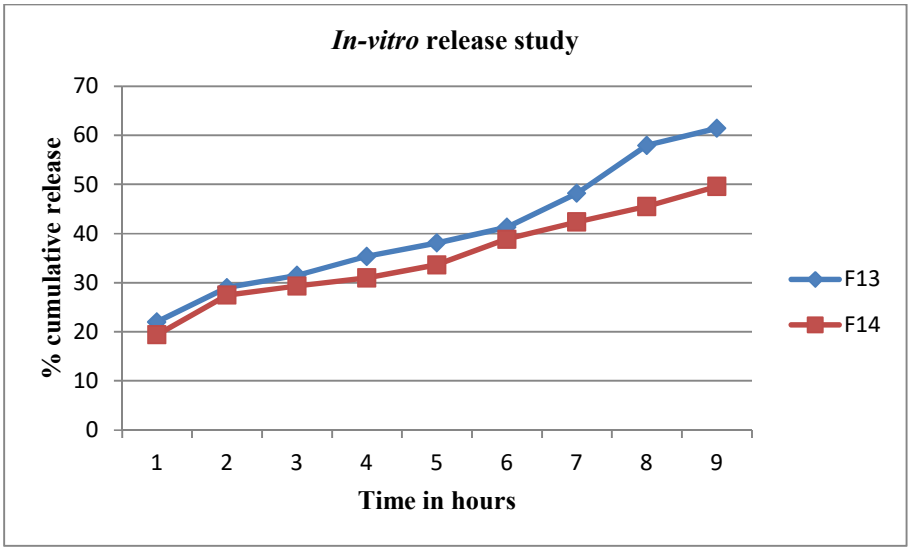


Figure15:*In-vitro* comparative diffusion profile of proniosomal formulations containing Metronidazole Benzoate (F_{13}&F_{14})

The *in-vitro* diffusion studies of all formulations were carried out using Franz diffusion cell with egg membrane as the semi-permeable membrane. The data obtained is given in table 11 & plots were drawn between % cumulative release & time as shown in the above figures. It was observed that the formulations showed percentage cumulative release in the range of 32.44-82.05% over a period of 8 hrs. The formulations made from span60 showed more release than the ones made from span40, tween 20&60. An increase in the cholesterol content showed an increase in drug diffusion. This may be attributed to the increased lipophilic nature of the proniosomal complex. F_{12} containing cholesterol: span60 in the ratio 1:1 was taken as the best formulation since it showed highest and optimum drug release over a period of 8 hrs. Replacement of the membrane stabiliser cholesterol by lecithin gave least dug diffusion. Hence it cannot be used as an effective membrane stabiliser.

Evaluation of optimised formulation

Optimised formulation F_{12} was further subjected to the following studies:

***Ex-vivo* skin permeation study**

Table 12: Results of *ex-vivo* skin permeation study

Time in hours	Cumulative % permeation
0.5	5.8
1	11.75
2	18.14
3	21.90
4	26.96
5	35.69
6	46.52
7	56.43
8	63.99
9	66.45
10	71.92
11	75.82
12	84.71

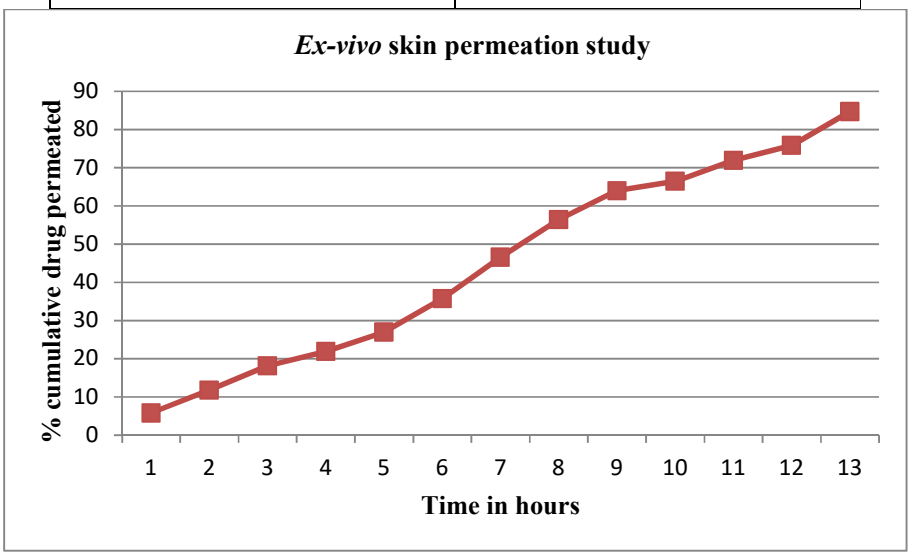


Figure16: Drug permeation profile of F_{12} formulation

The drug permeation of the formulation F_{12} through the abdominal skin of chicken was carried using Franz diffusion cell & the results are reported in table 12. The permeation profile was plotted between %cumulative drug permeated v/s time. It was observed that the formulation showed an optimum release of 84.71% over a period of 12 hrs.

Rate of spontaneity

The maximum benefit from the proniosome formulations can be speculated when abundant numbers of vesicles are formed after hydration. The number of vesicles formed per $mm^3$was found to be 4.

Vesicle size analysis

It was done using optical microscope at 100X magnification. The vesicular size was found to be 13.65±0.24µm which is in correlation with the results obtained from SEM.

Vesicular size by SEM

The surface morphology of the formulated proniosomal derived niosomal vesicles (formulationF_{12}) were confirmed by scanning electron microscopy. The vesicles are spherical in shape and smooth in nature.

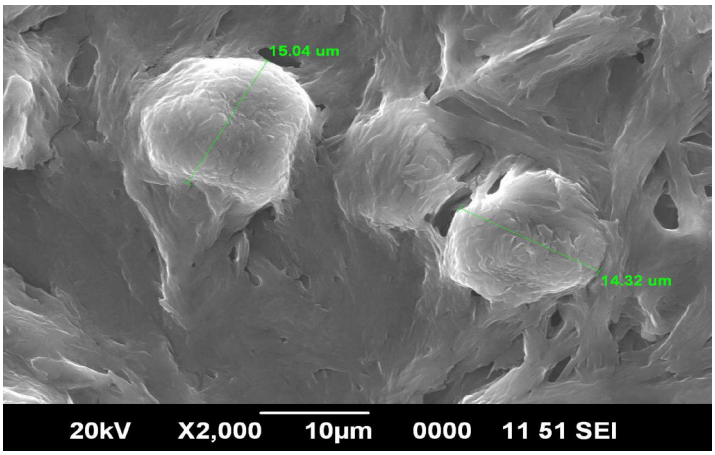


Figure17: Scanning electron microscopic image of Metronidazole benzoate loaded proniosomal derived niosomes(optimised formulation)

Antifungal study

The microbiological assay of Metronidazole benzoate pure drug & 12^{th} hour release sample solution of F_{12} proniosomal formulation was carried out .The diameter of the zone of inhibition was taken as a measure of the drug's antifungal activity. The zone of inhibition of

the pure drug & sample were found to be 26mm & 23mm respectively. Hence the results revealed that the developed proniosomal formulation is as effective as the pure drug in antifungal activity.

Figure18: Zone of inhibition obtained for Metronidazole benzoate proniosome (F_{12}) and pure drug using *Candida albicans*

Calculation of total drug loading

The formulation of the patch was made in such a way that each small circular patch of 1.25cm radius (which is the radius of the Franz diffusion cell) contains 10mg of the drug. The total amount of drug to be loaded in the patch was calculated by measuring the total area of the petri dish in which the patch will be casted. The calculation was done as follows:

* Area of the small circular patch = 4.91cm^2

* Desired drug content in the small patch =10mg

* Area of the petri dish = 67.89 cm^2

* Total amount of priniosomal powder to be loaded = 67.89 x 10 / 4.91 = 138mg

Hence 138mg of the optimised proniosomal formulation (F_{12}) was added in order to get 10mg per small circular patch.

PREPARATION OF TRANSDERMAL PATCH

As per the methodology transdermal patches were prepared using HPMC by solvent casting method. Firstly the polymer was dissolved in particular solvent then the proniosome formulation F_{12} was added into the solution. Plasticizer was added to the solution. This was casted on petridish. It was covered by funnel to control evaporation of solvent.

EVALUATION OF TRANSDERMAL PATCH

Physical appearance

All the transdermal films were visually inspected for colour, clarity, flexibility and smoothness.

Thickness

5 patches were prepared and the thickness was measured using Vernier calliper. All the prepared patches showed similar thickness. The average thickness was found to be 0.226mm.

Folding endurance

The folding endurance of transdermal patches were measured manually. This test is important to check the ability of sample to withstand folding. Evaluation of folding endurance involves determining the folding capacity of the film subjected to frequent conditions of folding. This also gives an indication of brittle nature, less folding endurance indicates more brittleness**.** The average folding endurance was found to be 43.

Percentage moisture loss

The average percentage moisture loss of the prepared patches was found to be 0.734%. The percentage moisture loss may be less due to the hydrophilic nature of the polymer.

Percentage moisture absorption

The average percentage moisture absorption of the prepared patches was found to be 4.69%. The percentage moisture absorption may be more due to the hydrophilic nature of the polymer.

Drug content determination

Drug content in a small circular patch was analyzed spectrophotometrically. Drug content of the patch was carried out to ascertain that the drug is uniformly distributed into the formulation. The prepared patch showed optimum drug content of 89.24%. Hence it can be concluded that the drug is uniformly distributed in the formulation.

Water vapour transmission rate

Water vapour transmission determines the permeability characteristics of the patch. The water vapour transmission rate of the prepared patch was found to be 1.55g.Hence the result reveals that the formulation is permeable to water vapour.

Percentage flatness test

Table13: Results of % flatness test

Initial length(cm)				Final length(cm)				% Constriction	% Flatness
Trial1	Trial2	Trial3	Avg	Trial1	Trial2	Trial3	Avg		
3	5	3	3.66	3	5	3	3.66	0	100

The flatness study showed that the patch had the same strip length before & after their cuts, indicating 100% flatness. Thus no amount of constriction was observed. This reveals that the patch had a smooth &flat surface & the smooth surface could be maintained when the patch was applied to the skin.

***In-vitro* diffusion study of transdermal patch**

Table14: Results of *in-vitro* release study of transdermal patch

Time in hours	%Cumulative drug release
0.5	6.7
1	10.83
2	17.25
3	21.9
4	25.76
5	34.03
6	45.29

Time in hours	%Cumulative drug release
7	54.43
8	63.99
9	65.69
10	70.18
11	74.82
12	80.71

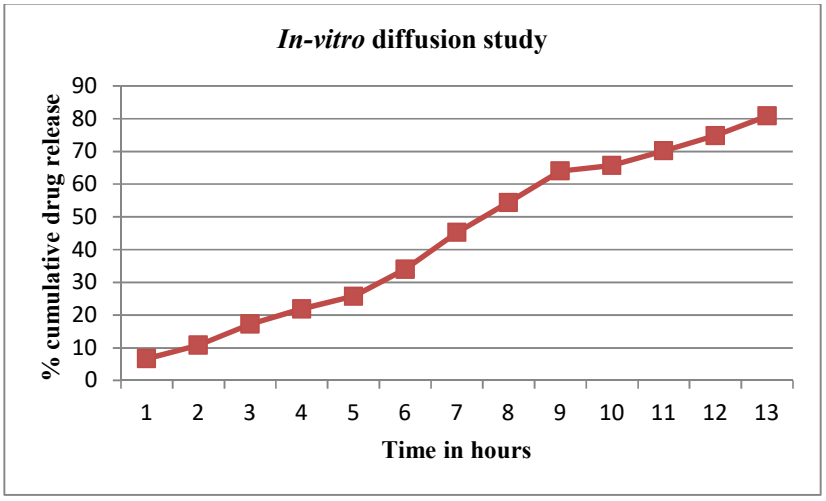


Figure19:*In-vitro* diffusion study of transdermal patch of optimised formulation

The *in-vitro* diffusion study of the transdermal patch incorporated with F_{12} was carried out using Franz diffusion cell with egg membrane as the semi-permeable membrane. The data obtained is given in table14 & plot was drawn between % cumulative drug release & time as shown in the above figure. The percentage cumulative release over a period of 12 hrs was found to be 80.71%.

Release kinetics

Table15: Data for kinetic study

Sl.no	Time(hrs)	% CDR	Log%CDR	% cumulative drug remaining	Log % cumulative drug remaining	Log time	$Time^{1/2}$
1	0.5	6.7	0.826	93.3	1.969	-0.30	0.707
2	1	10.83	1.034	89.17	1.950	0	1
3	2	17.25	1.236	82.75	1.917	0.301	1.414
4	3	21.9	1.340	78.1	1.892	0.477	1.732
5	4	25.76	1.410	74.24	1.870	0.602	2
6	5	34.03	1.531	65.97	1.819	0.698	2.236
7	6	45.29	1.656	54.71	1.738	0.778	2.449
8	7	54.43	1.735	45.57	1.658	0.845	2.645
9	8	63.99	1.806	36.01	1.556	0.903	2.828
10	9	65.69	1.817	34.31	1.535	0.954	3
11	10	70.18	1.846	29.82	1.474	1	3.162
12	11	74.82	1.874	25.18	1.401	1.041	3.316
13	12	80.71	1.907	19.2	1.283	1.079	3.464

In-vitro release data obtained for the trandermal patch containing optimised proniosome formulation was subjected to kinetic analysis. The percentage cumulative release data obtained were fitted to zero order, first order, Higuchi square root of time & Korsmeyer – Peppas equation to understand the mechanism of drug release from the proniosomal transdermal patch. The plots are shown below. From the regression values, it was concluded that the formulation followed zero order kinetics. The slope value (n) obtained from Korsmeyer- Peppas plot was 0.815, which indicates that the formulation followed non-Fickian mechanism of drug release.

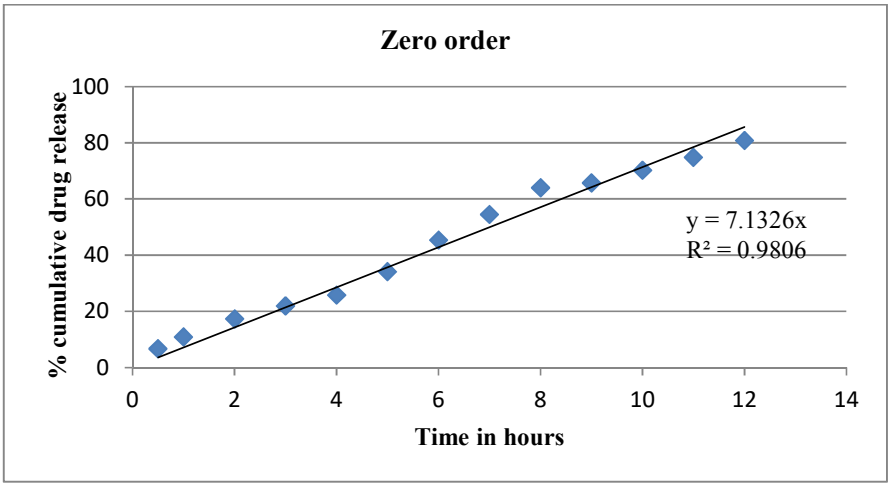


Figure20: Zero order plot

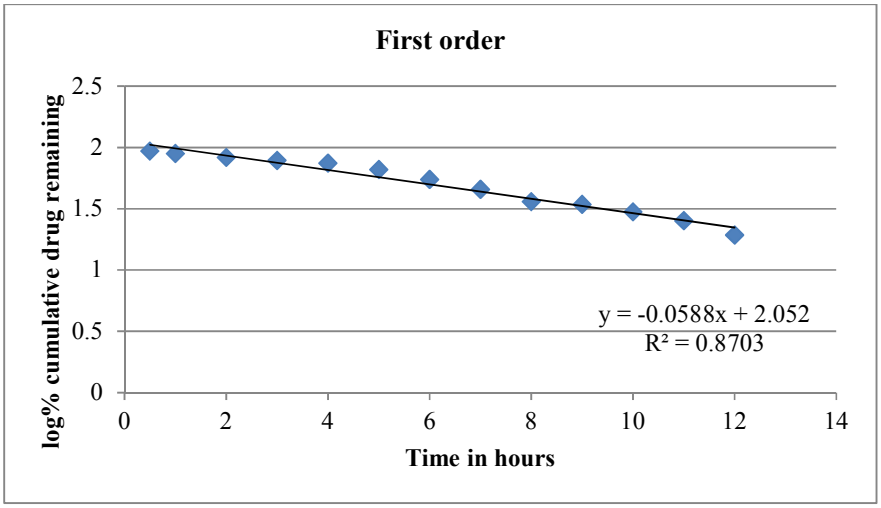


Figure21: First order plot

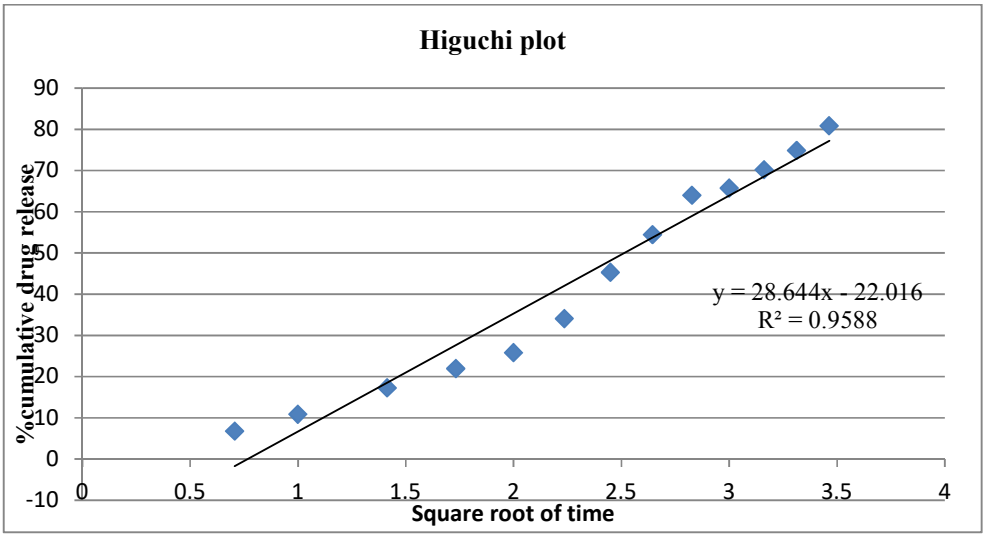


Figure22: Higuchi plot

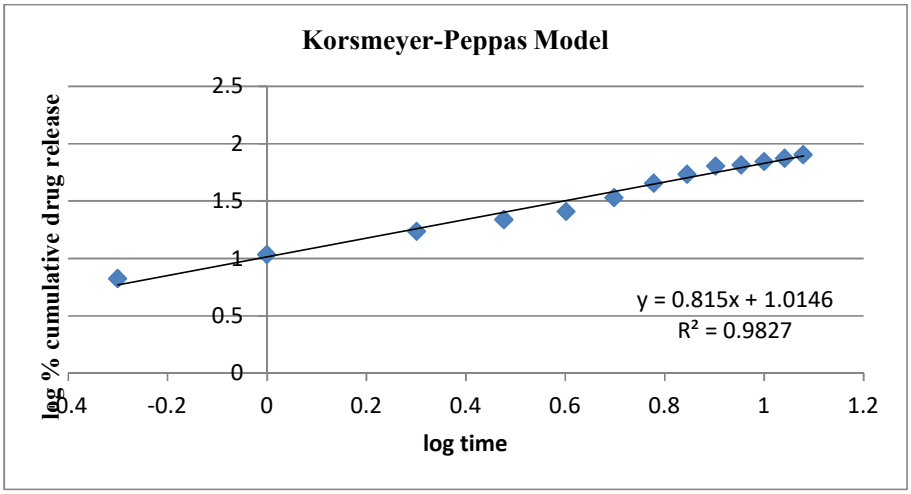


Figure23: Korsmeyer-Peppas plot

Table16: Result of kinetic data analysis

Formulation	Zero order	First order	Higuchi model	Korsmeyer-Peppas model	
F_{12} proniosomal patch	R^2	R^2	R^2	n	R^2
	0.9806	0.8703	0.9588	0.815	0.9827

Stability study

Stability study for the most satisfactory formulation F_{12} after incorporating into transdermal patch was carried out at accelerated temperature 40±2ºC and RH 75±5% for 45 days. After 45 days the patch was evaluated for physical appearance, drug content &*in-vitro* release study. From the results obtained it was observe that there was no major change in various parameters at accelerated temperature. Thus it can be concluded that the formulation is stable at the specified temperature for a period of 45 days.

Table17: Results of stability study

Sl.no	Accelerated temp: 40±2ºC, Relative humidity: 75±5% RH			
	Parameters	Initial	30^{th} day	45^{th} day
1.	Physical appearance	Yellowish white colour	No change	No change
2.	Drug content	89.24%w/w	89.00%w/w	88.62%w/w
3.	% Cumulative drug release	80.8%	80%	79.57%

SUMMARY AND CONCLUSION

Aim

In the present study, an attempt was made to formulate proniosomes containing Metronidazole benzoate and incorporating it in transdermal patch for controlled and prolonged release of drug. The drug has low aqueous solubility, which can affect its dissolution leading to poor BA. Incorporation into proniosomes can lead to reduced dose, improved bioavailability and prolonged release. Further formulating in form of transdermal patch allows controlled release of the drug.

Formulation

Proniosomes were prepared using slurry method with different non- ionic surfactants and also in different surfactant: cholesterol ratios. Two formulations were prepared using different excepients. In one formulation lecithin was used as the membrane stabilizer and in another the carrier used was lactose .From the prepared proniosomal formulations , the best formulation which contained surfactant:cholesterol in the ratio 1:1and mannitol as the carrier was selected based on various evaluation parameters and was incorporated into transdermal patch prepared using HPMC as the polymer. A total of 14 formulations were prepared.

UV spectrophotometric method was developed for determining Metronidazole benzoate in 5% ethanol at 309nm. A regression coefficient value of 0.9977 was noticed.

Evaluation

- FT-IR spectra of pure drug and drug-excepient mixture confirmed that there were no major interactions. Hence they were compatible.
- The proniosomal formulations were subjected to studies like angle of repose, optical microscopy, drug content analysis, entrapment efficiency and *in- vitro* diffusion study.
- The optimized formulation (F_{12}) was further subjected to studies like *ex-vivo* permeation, rate of spontaneity, vesicle size analysis, antifungal study and SEM.
- The prepared patch was evaluated for physical appearance, thickness, folding endurance, %moisture loss, % moisture absorption, drug content, water vapour transmission rate, % flatness, in-vitro diffusion study.

- Release kinetics data analysis revealed that the patch followed zero order kinetics with non-Fickian diffusion of drug.
- Stability studies were carried out at accelerated temperature 40±2ºC, 75±5 % RH for 45 days. There were no significant changes in the physical appearance, drug content and *in-vitro* drug diffusion profile.

CONCLUSION

Introduction of proniosomes have initiated a new area in vesicular research in transdermal drug delivery. Different reports show a promising future of proniosomes in making the topical delivery of various agents more effective. It was confirmed that proniosomal transdermal patch of metronidazole benzoate showed a better diffusion as well as stability profile , hence providing an attractive carrier for prolonged and controlled topical delivery. In conclusion , the novel proniosomal formulation is a promising option for transdermal delivery of metronidazole benzoate in the treatment of various systemic as well as topical bacterial infections. Further studies using animal models will throw more light on the effectiveness of the formulation.

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